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The Editorial Board will consider manuscripts dealing with all aspects of preclinical and clinical cancer treatment. These may include such topics as surgery, radiotherapy, chemotherapy, immunotherapy, supportive care, pharmacology, cell biology and kinetics, mechanisms of drug action, and medicinal chemistry.

The journal will consist of two parts. One part will consider previously unpublished manuscripts of original work under four main categories:

1. **Clinical or preclinical research.**
2. **Correspondence regarding manuscripts previously published in *Cancer Treatment Reports*. Letters on other topics will be published at the discretion of the Editorial Board.**
3. **Invited guest editorials and reviews. Unsolicited contributions will be considered on their individual merits.**
4. **Brief communications. Under this category the Editorial Board will consider short contributions which should not exceed five typewritten pages. Four types of communication will be considered in this category.**
   - **(a) Ideas relating to cancer treatment.**
   - **(b) Preliminary research reports.**
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All material submitted for consideration in the journal will be subject to review when appropriate by at least two outside reviewers and one member of the Editorial Board. The decision to publish a manuscript is solely the responsibility of the Editorial Board.

The second part of this journal will consist of supplements which may include proceedings of meetings and symposia and program information from the Division of Cancer Treatment, National Cancer Institute. Requests to publish information in *Cancer Treatment Reports* supplement will be considered by the Editorial Board.

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Begin the article with a short summary.

Text
Submit an original and three copies of the manuscript, typed double-spaced. Use accepted spelling, abbreviation, and hyphenation found in *Webster's New International Dictionary*. Footnotes should be typed at the bottom of the page on which they appear.

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Submit each table on a separate page, typed double-spaced. Please use standard abbreviations; otherwise spell out.

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Illustrations
Photographs and graphs preferably should be clear, glossy prints. Original art work should be done in black ink for good reproduction. Please send 2 copies of each print. Color photographs cannot be used.

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Names of compounds should conform to American usage. See *USAN and the USP Dictionary of Drug Names* or *The Merck Index* for proper nomenclature.

Abbreviations
Only standard abbreviations are acceptable. If a new or coined abbreviation is used, it must be defined when first mentioned.

Brief Reports
Manuscripts published in the Brief Reports section can be no longer than 5 double-spaced pages including references, tables, and a short summary; figures are usually omitted.

Letters to the Editor
Letters to the Editor can be no longer than 3 double-spaced text pages and can be submitted with no more than 1 table and 5 references; figures are usually omitted.

Clinical Trials Letters
Letters published in this section can be no longer than 300 words and can be submitted with no more than 1 table and 5 references, and no figures.

Symposia
Requests to publish the proceedings of symposia should be addressed to the Managing Editor. A copy of the agenda and a list of speakers should accompany such requests.

Submission of Manuscripts
Manuscripts and all inquiries concerning the status of manuscripts should be addressed to: Managing Editor, Cancer Treatment Reports, National Cancer Institute, 7910 Woodmont Ave, Rm 8C08, Landow Bldg, Bethesda, MD 20014 (301-496-6975). We invite all authors to submit with their manuscripts a short list of possible reviewers. The editorial board will take these names into consideration in selecting those who will be asked to evaluate your manuscript.

Reprints
Supplied free of charge: 250 reprints (50 reprints for Letters to the Editor) will be sent to the senior author about three weeks after publication of the article.

ANNOUNCEMENT

The readership has undoubtedly noticed that *Cancer Treatment Reports* has been published with increasing frequency over the last several months. This is due in part to the reorganization of the Publications Section into the new Scientific Information Branch within the Division of Cancer Treatment, which has aided in the streamlining of editorial office operations by resolving many of the administrative and support problems that have plagued the journal. The editorial staff has been able to turn its full attention to the backlog of work and anticipates that the journal will publish issues on schedule again in July of this year. Steps have also been taken to streamline the review process, which should expedite the processing of original manuscripts and decrease the frustrating time lag between submission and publication in the future. Because timely publication of the journal on a monthly schedule requires preparation of 1982 issues in the early fall, we will continue to publish *Cancer Treatment Reports* bimonthly throughout 1981. We have, however, acquired editorial support for the preparation and publication of symposia that were scheduled for publication in 1980. Because the number of regular issues has temporarily been reduced to six, the Government Printing Office has agreed to include the proceedings of symposia in this year's subscription cost. In the future, symposia will be edited and published separately. This should prevent a recurrence of our recent difficulties in the future.

In this issue, a new section of the journal is being introduced: "Clinical Trials Letters." Clinical Trial Letters enable us to publish data on clinical trials that have produced negative results. Because of their brevity, they can and will be processed quickly and published on a regular basis. In addition, we will provide a separate index of Clinical Trials Letters at the end of each year so that the negative clinical trials that have been performed in a variety of cancers can be easily summarized.

We appreciate the patience of the readership and the authors of accepted papers during this period of reorganization and we feel confident that timely publication of the journal will be maintained in the future.

Susan Molloy Hubbard
Jean Griffin Baum

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Third University of Arizona Cancer Center Conference on Human Tumor Cloning

The Secretary of Health and Human Services has determined that the publication of this periodical is necessary in the transaction of the public business required by law of this Department. Use of funds for printing this periodical has been approved by the Director of the Office of Management and Budget through January 31, 1983.

New Drugs in Ovarian Cancer and Malignant Melanoma: In Vitro Phase II Screening With the Human Tumor Stem Cell Assay[1,2]

Sydney E. Salmon,* Frank L. Meyskens, Jr, David S. Alberts, Barbara Soehnlen, and Laurie Young[3,4]

SUMMARY

The successful development of a soft agar clonogenic assay for human tumor stem cells provides an in vitro technique with a high degree of accuracy for predicting in vivo clinical response to standard anticancer drugs. We used this system to conduct an "in vitro phase II trial" in human ovarian cancer and melanoma. This approach can potentially identify active phase I–II drugs suitable for treatment of given tumor types for specific patients and eliminates the need to subject patients (who would be predicted not to respond) to toxic side effects. In vitro sensitivity for new agents was operationally defined as at least a 70% reduction of tumor colony-forming units (TCFU) at concentrations which are readily achievable pharmacologically. The new agents AMSA and vindesine (as well as vinblastine) appeared to have activity in melanoma, while PALA and thymidine were inactive. Pentamethylmelamine, mitomycin C, methyl-GAG, and AMSA were relatively ineffective in ovarian cancer. Vinblastine and vindesine had definite activity. The human tumor stem cell assay may thus provide the basis for a useful alternative to the current clinical phase II testing approach for identifying antitumor activity of new agents. Validation of this concept with correlative in vitro and in vivo phase II trials of new agents in patients with tumor types predicted to be sensitive is clearly warranted.

[Cancer Treat Rep 65:1–12, 1981]

Development of new drugs for ovarian cancer, malignant melanoma, or any other specific type of cancer has thus far been a very arduous and time-consuming procedure. The process, which initiates from rational design, serendipitous discovery, or random screening in a few signal mouse tumors has probably missed a number of compounds which were inactive in L1210 or P388 leukemia, but which would have had activity for other tumor types. Even using a broadened panel of five or six transplantable mouse tumors of different histopathologic types would be the conceptual equivalent of testing a new drug on five or six patients, each with a different type of cancer. After a new drug is found to be active in screening, it must pass preclinical and clinical toxicology trials before it can be brought to large-scale clinical phase II and III studies in various tumor types. Viewed in that context, it is perhaps remarkable that useful drugs have, in fact, been identified. In fact, many drugs have been identified through other mechanisms in various countries..

[1]Received Feb 5, 1980; revised May 1, 1980; accepted May 12, 1980.

[2]Supported by Public Health Service grants CA-21839, CA-17094, and CA-23074 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3]University of Arizona Cancer Center, Tucson.

[4]We thank Dale Curtis and Dave Listowski for computer graphics assistance; George Chen for correlative data assessment; Thomas Moon for statistical analysis of the data; Reea Rodriguez for secretarial assistance; and William Casey, H. Jack Smith, Earl Surwit, and other clinical colleagues for patient referral.

*_Reprint requests to:_ Sydney E. Salmon, MD, Rm 6335, Cancer Center Division, University of Arizona, 1501 N Campbell Ave, Tucson, AZ 85724.

We believe that the recent development of in vitro soft agar colony assays for human tumor stem cells (1–10) shows significant promise of shortening and simplifying the entire drug testing procedure including the preclinical drug screening for active compounds, clinical trials of new agents, and the final selection of treatment for individual patients. These comments are particularly germane for ovarian cancer and malignant melanoma because they are readily grown in the tumor stem cell assay, starting with either malignant effusions or solid tumors (3,5). Our initial approach to drug testing with the stem cell assay in ovarian cancer and melanoma was to use drugs with known activity in patients and correlate in vitro and in vivo sensitivity (7). These correlation studies continue to be quite encouraging (8,10). However, our central focus here will be the examination of new phase I–II agents to determine the frequency with which significant inhibition of ovarian and melanoma tumor colony-forming units (TCFU) is observed at pharmacologically achievable doses. In that sense, we are conducting an "*in vitro phase II trial*" of new anticancer drugs. Additionally, we have applied the assay to make some pharmacologic comparisons of 1-hour versus continuous contact of TCFU to a drug to evaluate potential schedule dependency of some of these agents.

METHODS

Tumor biopsies and malignant effusions were obtained from patients with adenocarcinoma of the ovary or melanoma. Techniques for preparing single-cell suspensions, incubating drugs, and plating the cells in agar culture were reported previously from our laboratory (3,5,7,8), except that conditioned medium was not used, since tumor colony growth was obtained without it. In our standard drug assay system, cells are exposed to various concentrations of drugs in tissue culture tubes for 1 hour at 37°C in McCoy's 5A medium with 10% heat-inactivated fetal calf serum, then washed and plated in the upper layer of the two-layer agar cultures. New agents studied included mitomycin C, vindesine, AMSA, dihydroxyanthracenedione, pentamethylmelamine (PMM), PALA, methyl-GAG, and thymidine. Dose-finding studies for new agents for which pharmacokinetic data were not available were carried out over a 3-log concentration range from 0.1 to 10 μg/ml. In the present study, some samples were also plated in the presence of standard concentrations of stable drugs incorporated into the agar. In most cases, 5×10^5 cells were plated in each 35-mm Petri dish. Freshly plated cultures were examined by inverted light microscopy to ascertain that aggregates were not present. Plates were cultured at 37°C in a humidified incubator with 5% CO_2 in air. Tumor clusters were apparent within 3–4 days. Ovarian tumor colonies usually were present in sufficient numbers and size to be counted 7–10 days after plating, while melanoma colonies could be counted after 10–14 days. Representative plates were prepared for morphologic analysis using our recently described dried-slide technique with Papanicolaou staining (9). Data from all experiments were entered into disc storage on a Wang 2300 laboratory computer which was used for data analysis and graphic output. Criteria for in vitro sensitivity for standard drugs were based on calculation of the area under linear survival-concentration curves and on ranking relative areas using results from an initial training set of patients for whom in vitro and in vivo studies were done (7,10). For new drugs with pharmacologic parameters that were less certain, we used an *operational* definition of sensitivity: ie, at least a 70% reduction in survival of TCFU at a relatively low dose of the drug. In all instances where pharmacokinetic data were available, the dose to achieve at least a 70% inhibition had to be $< 10\%$ of the maximal clinically achievable concentration $\times$ time ($C \times t$) product to designate a drug as being sensitive in vitro.

RESULTS

Relation of Drug Schedule to Lethality on Clonogenic Tumor Cells

Studies comparing 1-hour exposure of cells to drugs before plating versus continuous contact, in the agar, to drugs thought to be stable in vitro have been only recently initiated in our program. Figure 1 summarizes comparative time exposures for ovarian cancer cells to vinblastine. For the two patients studied in this system, a somewhat greater in vitro lethality was observed with continuous contact to vinblastine for the entire culture period (approximately 10 days) in comparison to the 1-hour exposure. However, in view of the fact that the effective $C \times t$ product for continuous contact would be > 200 times that for the 1-hour exposure, it would appear that prolonged contact was relatively ineffective. Further studies will be required to clarify this phenomenon.

Individual Agents

A study of a number of anticancer drugs which have not been extensively utilized in ovarian cancer or melanoma or are currently investigational agents (IND status) is now under way in our laboratories.

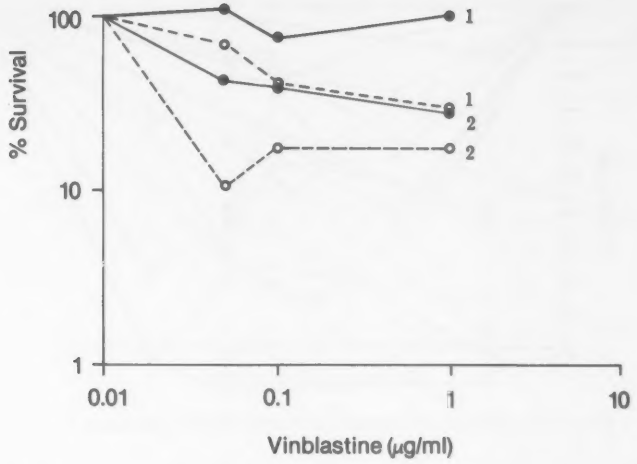


FIGURE 1.—Schedule-dependent TCFU inhibition by vinblastine in vitro: comparison of 1-hr exposure (—●—) and continuous contact (- - -○- - -) of ovarian tumor colony-forming cells from 2 patients (Nos. 1 and 2) to vinblastine.

Table 1 and figures 1–9 summarize the in vitro phase II investigations.

Mitomycin C

Mitomycin C is one of the standard anticancer agents that are only rarely used for early or advanced ovarian cancer. We investigated mitomycin C-induced lethality on tumor samples from 11 patients with ovarian cancer. The results of these studies (fig 2) indicate that for only one of these 11 patients, TCFU survival was reduced to < 30% of the control at pharmacologically achievable doses of mitomycin C. However, four of the 11 patients had steep curves to the 0.1-μg/ml dose level. Most of the patients who were studied were in relapse from prior alkylating agent therapy.

TABLE 1.—Sensitivity of TCFU to 1-hr exposure to single drugs*

Drug	No. of sensitive cultures/ No. tested	
	Ovarian cancer	Melanoma
Mitomycin C	1/11	NT†
PMM	2/5	NT
Vindesine	1/11	2/12
Methyl-GAG	2/11	0/5
PALA	0/8	0/5
AMSA	2/17	5/16
Dihydroxyanthracenedione	0/1	NT
Thymidine	NT	0/7
Vinblastine	1/7	NT

*For the purposes of this comparison, our operational definition of sensitivity is defined as a > 70% reduction in survival at 10% of the pharmacologically achievable 1-hr C × t.

†NT = not tested.

Pentamethylmelamine

Figure 3 summarizes our experience with the in vitro assay of the new agent PMM. This drug was formulated as an iv preparation because hexamethylmelamine (HMM) lacks sufficient solubility for iv use (11). PMM is under phase I–II study in several centers and is of interest in ovarian cancer because HMM is known to be active in this disease. Cells from two of the five patients studied thus far have shown a 70% reduction in survival of TCFU compared with the control. None of these patients had received HMM or PMM clinically. While one of these assays showed diminishing effect at increasing dose levels, this was not observed for the other patients studied. These preliminary data would suggest that PMM may prove to be an active drug for use in ovarian cancer treatment. Selection of this agent rather than HMM will, in part, depend on comparative toxicology and clinical pharmacology studies with both drugs. Since the activity of both HMM and PMM may be due to an intermediate product (12), the cells in the tumor suspensions appear to be capable of metabolizing PMM to the active form.

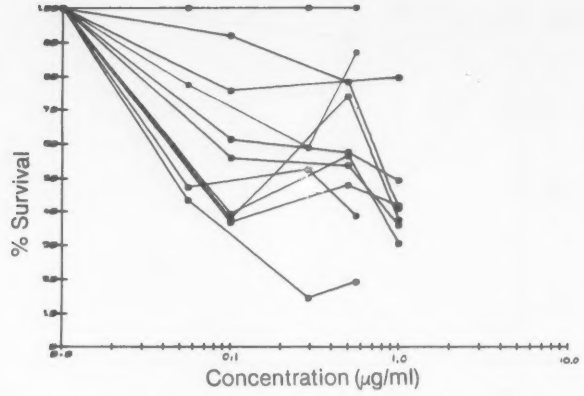


FIGURE 2.—Effect of 1-hr exposure of ovarian TCFU to mitomycin C.

Vindesine

The vinblastine analog vindesine (13) has been studied in vitro against TCFU from 11 patients with ovarian carcinoma (fig 4A) and from 12 with melanoma (fig 4B). Figure 4A shows comparative lethality of vindesine and vinblastine on ovarian tumor clonogenic cells from eight patients. When the two drugs were thus studied simultaneously in vitro, there appeared to be no clear advantage to vindesine over vinblastine. Reduction in survival to < 30% of the control TCFU at the 0.1-μg dose in the 1-hour exposure experiments was observed for one of the 11 patients with ovarian cancer (not all depicted in fig 4A) and for two of the 12 with melanoma (fig 4B). We have observed occasional excellent objective responses in patients with drug-refractory ovarian cancer treated with vinblastine (two partial remissions) and in patients with drug-refractory melanoma treated with vindesine (one partial remission) which were predicted by the stem cell assay.[5] Based on the comparative in vitro stud-

[5]Alberts DS, Salmon SE, Chen HSG, et al. Predictive chemotherapy of ovarian cancer using an in vitro clonogenic assay. Manuscript in press in *Lancet*.

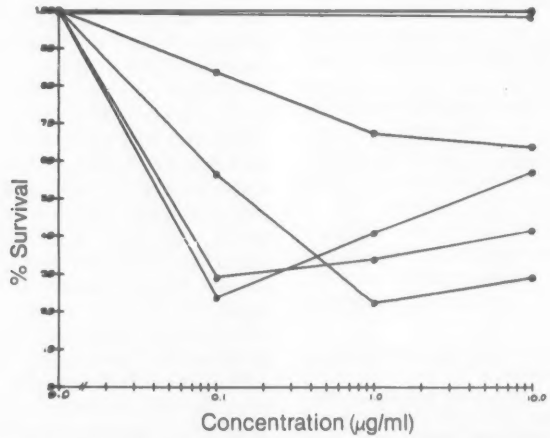


FIGURE 3.—Effect of 1-hr exposure of ovarian TCFU to PMM.

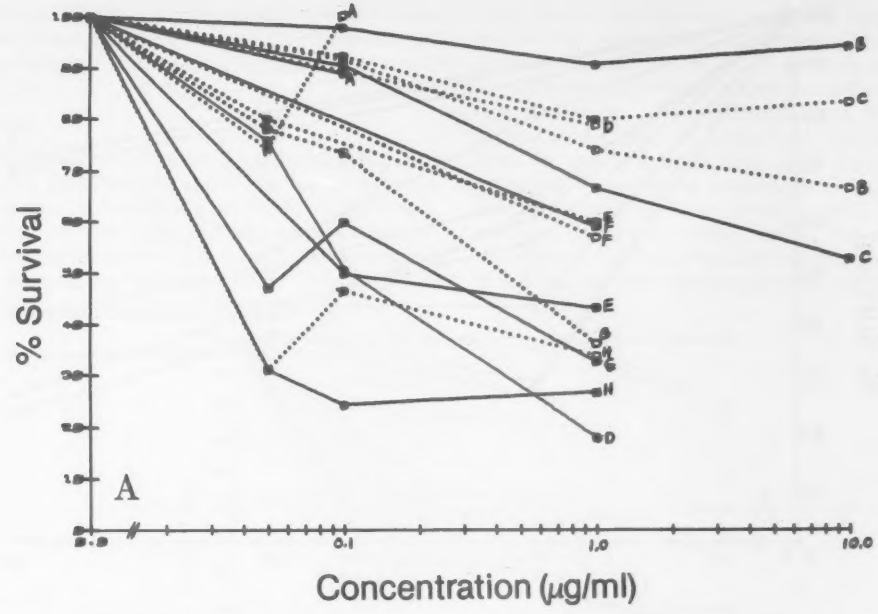
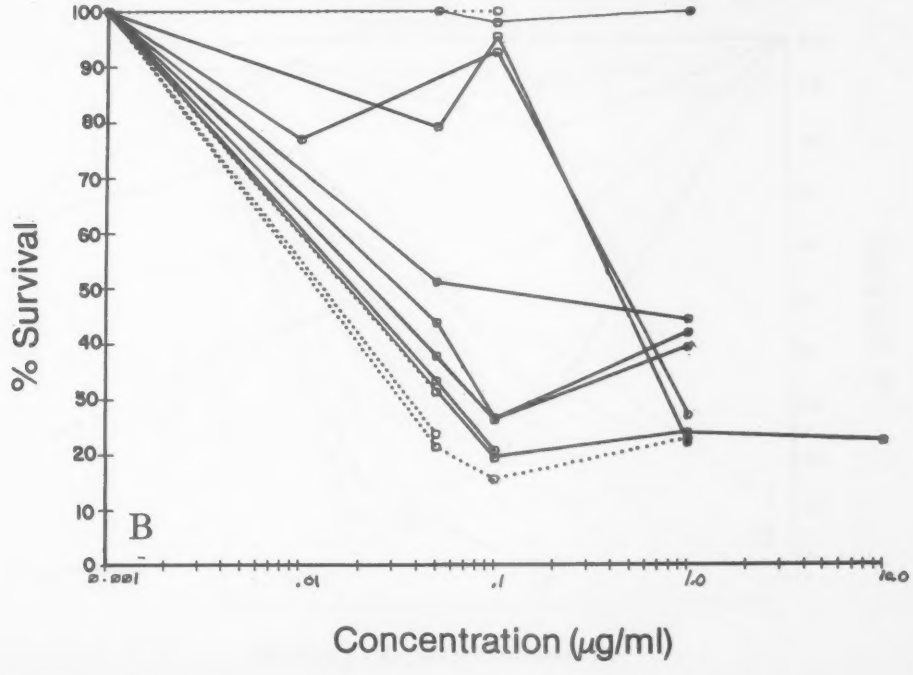


FIGURE 4.—(A) Comparative lethality of 1-hr exposure of ovarian TCFU to vindesine (————) or vinblastine (· · · · · ·). (B) Effect of vindesine on melanoma TCFU. ———— = 1-hr exposure; · · · · · · · = continuous contact.



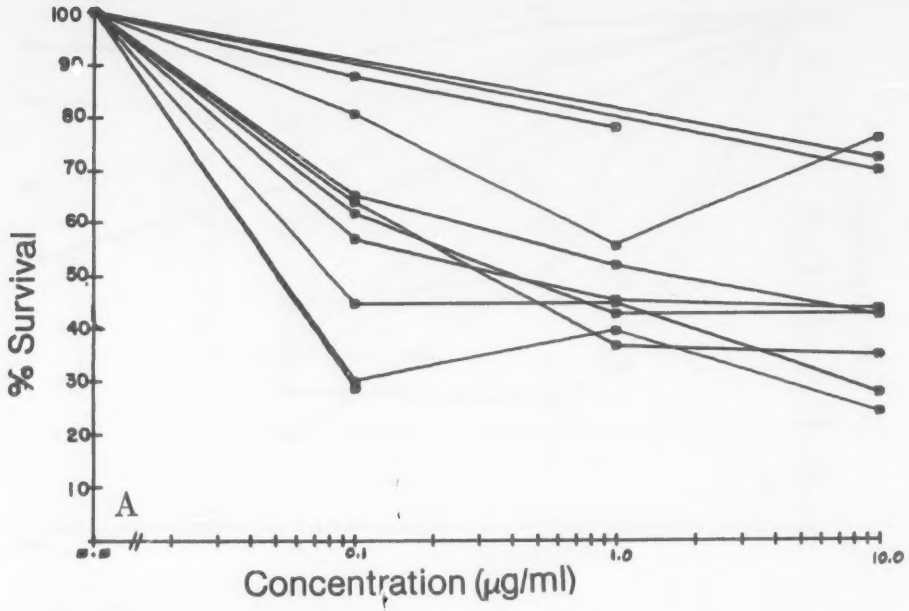
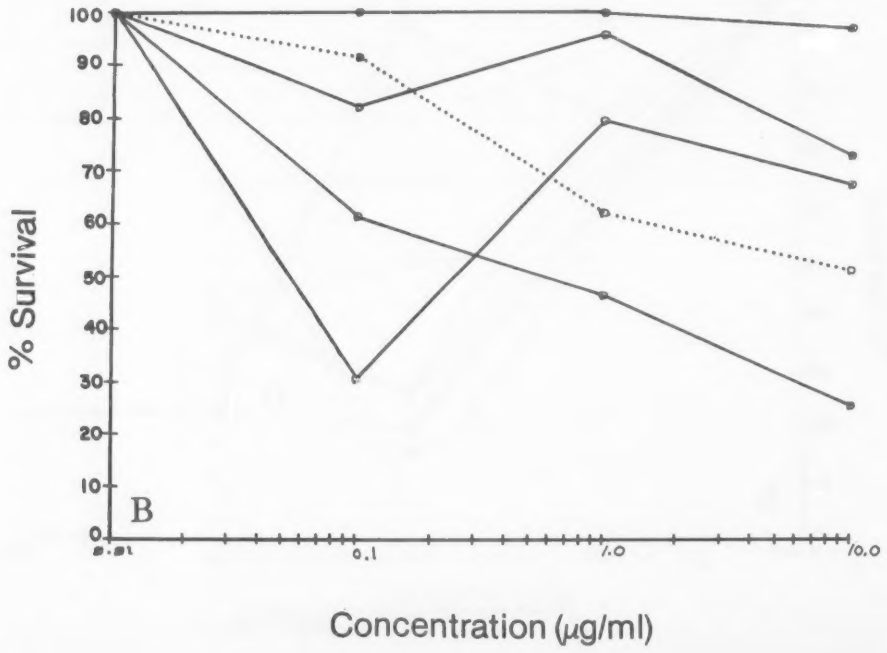


FIGURE 5.—Effect of exposure of TCFU to methyl-GAG (———— = 1-hr exposure; · · · · · · = continuous contact): (A) ovarian carcinoma; (B) melanoma.



ies in ovarian cancer we anticipate that vindesine and vinblastine may have comparable clinical effects in drug-resistant patients if the pharmacokinetics are similar.

Methyl-GAG

Methyl-GAG, an inhibitor of polyamine synthesis, was tested against tumor stem cells from 11 patients with ovarian carcinoma (fig 5A) and from five with melanoma (fig 5B). This long-standing investigational agent has recently been revived and found active in renal and bladder cancer (14). In our in vitro assays, methyl-GAG had only intermediate lethality (or less) in cells from most of the patients studied, and survival of TCFU for only two of the 11 patients with ovarian cancer was reduced to 30% of control at the 0.1-μg/ml dose in vitro. While some increasing lethality was observed with increasing dose, it seems unlikely that such high doses of methyl-GAG would be achievable in vivo without excessive toxicity. Prior studies with high-dose methyl-GAG (in the 1960s) led to the premature abandonment of this drug. Our studies suggest that occasional patients with ovarian cancer (ie, 10%–20%)

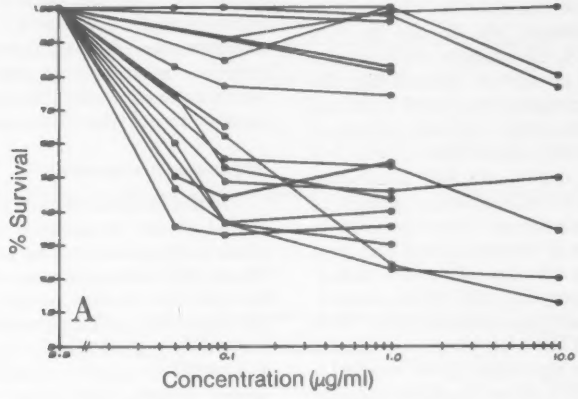
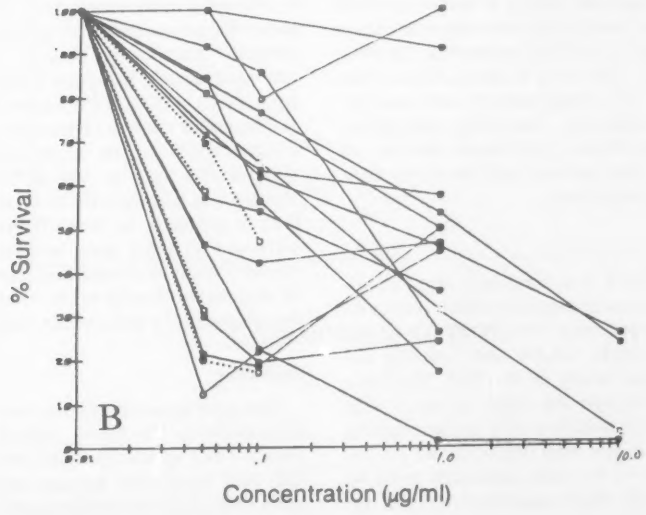


FIGURE 6.—Survival of TCFU after in vitro exposure to AMSA. ——— = 1-hr contact; · · · · · · = continuous exposure): (A) ovarian carcinoma; (B) melanoma.



may respond to methyl-GAG, but that patients with melanoma are less likely to respond.

AMSA

Promising preclinical results in a variety of tumors and acceptable phase I investigations of toxicity (15) have led to broad phase II trials with AMSA. In our clinical investigations we have seen significant activity of AMSA against a spectrum of tumors, including breast cancer, lymphoma, and melanoma (16). While animal studies suggested that this agent is cross-resistant with doxorubicin, some clinical observations in acute leukemia suggest that the drug may prove useful in patients who relapse after doxorubicin treatment (16). In our in vitro phase II study of AMSA, 17 patients with ovarian carcinoma (fig 6A) and 16 with melanoma (fig 6B) were studied at doses ranging from 0.05 to 10 μg/ml for 1 hour. We consider that 1 μg hour exposure should be pharmacologically achievable in vivo. Additionally, cells from four patients with melanoma were studied in vitro with continuous exposure to AMSA in the agar. Substantial heterogeneity in response to this agent was observed, and only two patients with ovarian carcinoma had a 30% reduction in cell survival compared to 30% of the control. However, five patients with melanoma had a > 70% reduction of TCFU with the 1 μg hour exposure to AMSA, which suggests that this agent would be active in melanoma. TCFU from an additional four patients with melanoma, studied with continuous contact to AMSA, showed no marked increase in lethality, suggesting that resistance may have more of a cellular uptake or biochemical basis than a cytokinetic one. Inasmuch as AMSA is known to bind to melanin granules, even a 1-hour exposure may provide "continuous contact" in melanoma. As with many of our other in vitro drug studies, AMSA was evaluated mainly in cells from patients who had received prior chemotherapy, including alkylating agents and anthracyclines. Additional studies on cells from patients who had not had previous drug exposure would be important.

PALA

The new agent PALA is a transition-state inhibitor of aspartate transcarbamoylase which blocks de novo pyrimidine biosynthesis (16–19). The drug has undergone phase I study (18,19) and recently entered phase II clinical trials in the US. We have completed in vitro studies for eight patients with ovarian cancer (fig 7A) and five with melanoma (fig 7B). A broad dose range was investigated (1–100 μg/ml), with 1 hour of in vitro exposure prior to culture. The highest in vitro concentration (100 μg/ml) approximates the plasma concentration attainable after a maximally tolerated clinical dose of 7.5 g/m². At exposures to relatively high in vitro doses, the TCFU of three of eight patients with ovarian cancer had survival reduced to < 30% of the control. Whether such high concentrations are achievable intratumorally remains to be established. Survival of TCFU for all of the five patients with melanoma was > 30% of the control even at these high doses, although one patient's clonogenic cells did manifest a sharp drop in survival at the highest dose tested (fig 7B). In our predictive studies with standard cytotoxic drugs, we found that, for in vivo activity, drugs had to exhibit substantial activity at 5%–10% of the clinically achievable C × t product of plasma concentration (7). If similar in vitro and in vivo dose-response relationships also apply to PALA, we would anticipate that this agent should be relatively inactive in ovarian cancer and in melanoma.

Dihydroxyanthracenedione

Certain anthraquinones have been studied as potential model compounds analogous to anthracyclines such as doxorubicin and daunorubicin (20,21). These new compounds have been designed with the thought that cardiac toxicity might be averted and therapeutic efficacy enhanced. Recently, a bis(hydroxyethylamino-ethylamino) anthraquinone was found to possess antitumor activity in several mouse tumors (21). The new agent dihydroxyanthracenedione has recently entered phase I clinical trial in San Antonio, TX, and Tucson, AZ. In conjunction with our phase I trial, we have carried out preliminary observations of the in vitro lethality of dihydroxyanthracenedione in the tumor stem cell assay for seven patients with breast or gynecologic cancers. Results of these in vitro survival curves are summarized in figure 8. Survival of TCFU was reduced to < 30% of the control for one patient with endometrial cancer (1-hour exposure) and for one with ovarian cancer (continuous exposure). Pharmacokinetic data as well as the results of phase I studies will be required to determine the achievable C × t products in vivo. However, in this pilot in vitro study, a log dose response covering doses of dihydroxyanthracenedione from 0.1 to 100 μg hour of exposure should more than bracket the pharmacokinetically achievable dose exposure in vivo.

Thymidine

The new agent thymidine is a naturally occurring nucleotide in the blood, which has been shown to inhibit DNA synthesis when given in excess amounts (22) and to inhibit human tumor growth in nude mice (23). At concentrations of ≥ 1 mM (242 μg/ml),

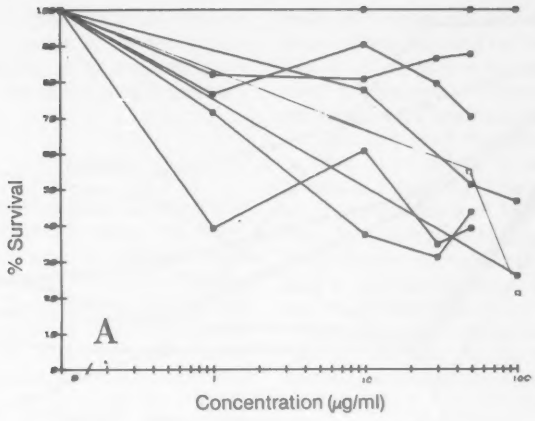
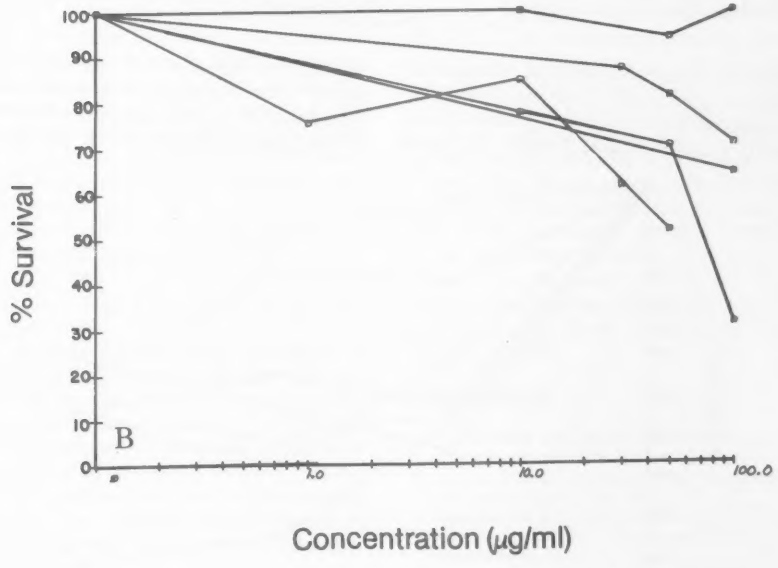


FIGURE 7.—Survival of TCFU after 1-hr exposure to PALA: (A) ovarian cancer; (B) melanoma.



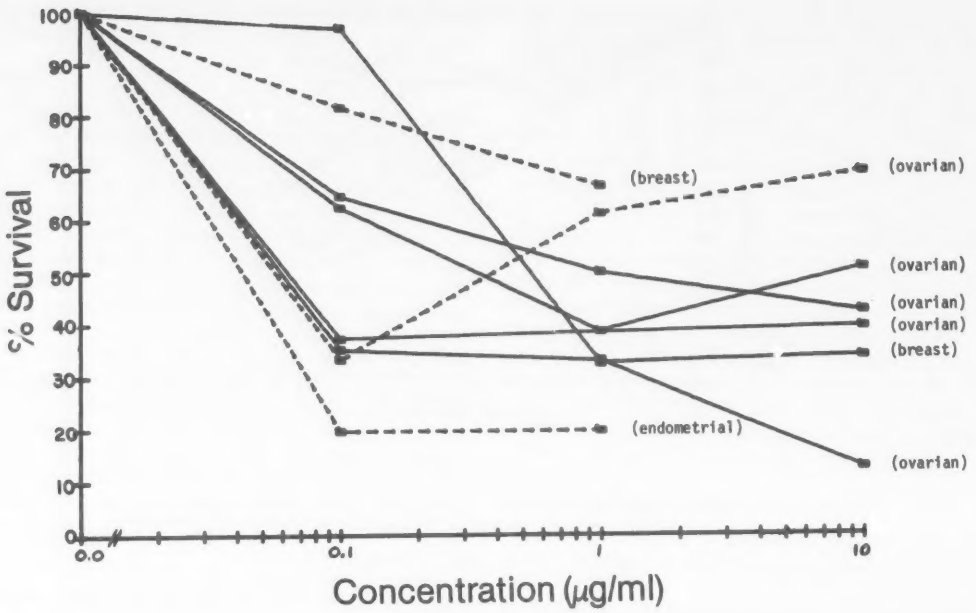


FIGURE 8.—Preliminary studies of 1-hr exposure (- - - - - -) and continuous contact (————) with dihydroxyanthracenedione on survival of gynecologic TCFU.

thymidine inhibits cellular proliferation and appears to have selective cytotoxic effects on neoplastic cells (22,23). This compound has undergone phase I study (24) and recently entered phase II trials. We have studied the effect of a broad dose range (50–1000 μg/ml) with 1-hour in vitro exposure to culture of cells from seven patients with melanoma (fig 9). One patient had reduction of TCFU to < 30% of the

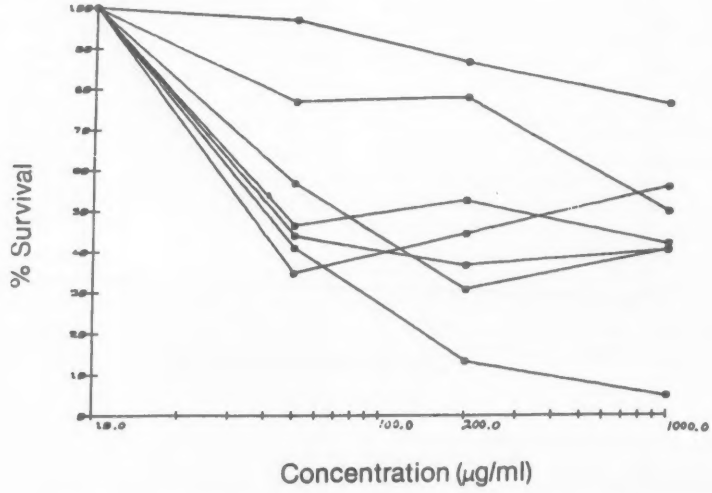


FIGURE 9.—Survival of melanoma TCFU after 1-hr exposure to thymidine.

control at a relatively high thymidine concentration (200 μg/ml). For the other patients, the plateau in survival seen with increasing drug concentration suggests that continuous exposure would not necessarily increase lethality.

DISCUSSION

In this investigation of in vitro phase II screening with the human tumor stem cell assay, we have focused on some of the new agents which are currently undergoing clinical trials in ovarian cancer and malignant melanoma. The feasibility of carrying out such in vitro studies rapidly on multiple agents is confirmed by our studies, inasmuch as we have been able to conduct simultaneous studies on these agents over the past year. It obviously has not yet been determined whether our in vitro results will correlate with the efficacy of these agents when given to heavily pretreated patients with ovarian cancer or melanoma. However, we have recently analyzed the in vitro effect of AMSA for ten patients with a variety of malignancies. Seven patients with a < 70% reduction in TCFU failed to respond while three patients with a > 70% reduction had a partial remission (16). Of greater interest in the future will be the application of this assay system to ovarian tumor and melanoma stem cells from patients who have received no prior treatment. When such data become available in assays carried out simultaneously with tests of standard agents known to be active (eg, melphalan and doxorubicin for ovarian cancer and actinomycin D and DTIC for melanoma), it might be possible to rank these drugs more realistically and to predict potential in vivo utility of new versus standard agents in such patients.

Our limited investigations of continuous contact versus 1-hour exposure for several drugs suggest that longer exposure may not offer an advantage. Clearly, this phenomenon will warrant further investigation in defining optimal drug exposures as well as in validating such observations through correlative clinical trials. The data which we have obtained thus far correlating the results of 1-hour exposure to standard anticancer drugs with results of clinical trials continue to be quite promising (10,25), and it is likely that studies from many other centers will also examine this question independently in ovarian cancer, melanoma, and other cancers over the next few years.

While many of the new phase I–II agents which we tested showed heterogeneity of response on TCFU from different patients with ovarian cancer and melanoma, it must be recognized that we have been carrying out "secondary" screening of these agents. That is, the drugs were developed on the basis of animal models employing leukemias and other neoplasms and not because they had any particular utility in ovarian cancer or melanoma. In at least one instance, we have advanced a drug to clinical trial on the basis of our in vitro sensitivity studies. We observed that 13-*cis*-retinoic acid is active in vitro in a variety of neoplasms including melanoma (5). Previously this agent had been considered as an "antipromoter" for cancer prevention rather than as a therapeutic agent. It will be of considerable interest to determine whether 13-*cis*-retinoic acid is therapeutically active and whether activity can be predicted in vitro for specific patients.

Recently, the National Cancer Institute, Bethesda, MD, has decided to make a major test of the human tumor clonogenic cell assay to determine whether it will be useful for primary drug screening of new compounds sent to the Institute for screening evaluation. The assay will thus be tested as an alternative to conventional screening in P388 leukemia and other in vivo mouse tumor systems. We believe that in primary screening, "positives" as defined with this assay should be identified when biopsy samples from the majority of a series (ie, > 50%) of untreated patients with a given tumor type manifest substantial lethality with the new agent tested. At that point, the new drug could be introduced into toxicology testing followed by clinical trial for the appropriate tumor type. We anticipate that ovarian cancer could be a signal neoplasm in the new drug screening effort (because it can be grown so readily). Melanoma, although slightly less readily grown, exhibits substantial heterogeneity (even among the clonogenic cells from each patient), and could be an excellent test system for this tumor type which is resistant to most drugs identified as active in murine systems. It seems reasonable to expect that the assay will detect new structures with more consistent efficacy in ovarian cancer and melanoma than those compounds which are currently available.

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Phase II Study of Chlorozotocin in the Treatment of Advanced Colorectal Cancer[1,2]

J. O. Lawton, G. R. Giles,* J. Hall, A. MacAdam, R. Hall, T. Matheson, and G. Bird[3]

SUMMARY

In 39 patients with advanced colorectal cancer and no previous chemotherapy, treatment with chlorozotocin was associated with a 5% objective response rate and a median survival of 19 weeks. All of the responses occurred in patients with rectal tumors. Mild gastrointestinal symptoms affected 64% of the patients after treatment, but bone marrow suppression was not evident. Chlorozotocin is not effective against colorectal cancer when used as a single agent given as an iv bolus at doses of 120 mg/m² (30 patients) and 150 mg/m² (nine patients). However, further studies using a higher dose might be worthwhile.

[Cancer Treat Rep 65:13–15, 1981]

The chloroethyl nitrosoureas, CCNU, BCNU, and methyl-CCNU, are recognized as effective antitumor agents. Structure-activity analysis suggests that the alkylating activities of nitrosoureas are responsible for the antitumor effects and that the associated myelotoxicity correlates with their carbamoylating propensity (1). Therefore, when chlorozotocin, a chloroethyl nitrosourea, was found to possess high alkylating and low carbamoylating activity (2), it was hoped to have an improved therapeutic index compared with that of the more established nitrosoureas.

Preliminary studies with chlorozotocin showed activity against L1210 and P388 leukemias and B16 melanoma, though it had only minimal activity against Lewis lung carcinoma (3). In addition, comparative studies with BCNU on bone marrow stem cell colonies showed relative sparing of marrow stem cells by chlorozotocin (4). Phase I studies from Georgetown University, Washington, DC, evaluated the drug at doses up to 175 mg/m² given to 35 patients.[4] They recorded two responses at a dose of 120 mg/m², both in patients with reticuloses. Leukopenia was not found to be a problem, but there was evidence of dose-related thrombocytopenia and mild gastrointestinal toxicity. With in vitro evidence that a dose of 120 mg/m² of chlorozotocin possessed equivalent alkylating activity to therapeutic doses of BCNU, the Georgetown group recommended phase II testing to begin at a dose of 120 mg/m². This study reports the results of a phase II trial of chlorozotocin in a group of patients with advanced colorectal cancer.

MATERIALS AND METHODS

Thirty-nine patients with histologic evidence of advanced adenocarcinoma of the colon or rectum were entered in the study. Each patient had a measurable malignant lesion which was used as an objective indicator of response to therapy. All patients were ambulatory with a Karnofsky performance status of ≥ 50 (22 patients had a rating ≥ 80) and an estimated survival time of at least 8 weeks. Exclusions from entry in the study included evidence of myelosuppression (wbc count $< 3500/mm^3$ and platelet count $< 110 \times 10^3/mm^3$), significant im-

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[4]Personal communication. Minutes of the Phase II Working Group, Georgetown University Studies, 1977.

pairment of renal or hepatic function, or prior treatment with chemotherapy or irradiation.

Chlorozotocin was supplied in powder form by the Division of Cancer Treatment, National Cancer Institute (Bethesda, MD), in 50-mg vials. This was reconstituted with sterile water to a concentration of 2.5 mg/ml immediately prior to administration and was given as a single iv bolus injection at a dose of 120 mg/m^2 for the first 30 patients; it was increased to 150 mg/m^2 for the last nine patients.

Before starting chemotherapy, all patients had a complete physical examination and laboratory evaluation including cbc, serum electrolytes, liver function tests, 24-hr creatinine clearance, and chest roentgenogram. On the day of therapy a cbc was obtained, and the patient's symptomatic status, performance status, and size of indicator lesion were recorded. Patients were treated on an outpatient basis, had regular followup to assess symptomatic and hematologic toxicity, and were re-evaluated at 6 weeks for evidence of response to therapy.

Response was defined using the Southwest Oncology Group criteria. An objective regression was recorded if there was a reduction by at least 50% of the sum of the largest perpendicular diameters of the indicator lesion, or if the liver was the indicator, a reduction of the sum of measurements below the costal margin at the midclavicular lines and xiphoid process by at least 30%, with improvement of all pretreatment abnormalities of liver function. Disease progression was recorded if there was an increase in size of the indicator lesion, evidence of any new area of malignant disease, or significant deterioration in symptomatic or performance status. Stable disease was recorded if there was no objective evidence of change in the malignant process. If, at re-evaluation, there was objective evidence of stable disease or regression, an additional course of chlorozotocin was given. Patients with disease progression were treated symptomatically or with alternative chemotherapy at the clinician's discretion, although one patient with subjective improvement but objective disease progression received additional chlorozotocin. Details of age, sex, and primary tumor of the patients in this study are shown in table 1, and the nature of the indicator lesions is shown in table 2.

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Males:females	26:13
Median age in yrs (range): 63 (53–77)	
Primary tumor site	
Rectum	18
Colon	21
Differentiation	
Well/moderate	35
Poor	4

TABLE 2.—Nature of indicator lesion

Indicator	No. of patients
Abdominal mass	8
Liver	
Palpable mass	18
Scan	6
Pulmonary metastases	6
Lymph nodes	1

RESULTS

A total of 62 courses of chlorozotocin were administered, with nine patients receiving a total of 18 courses at the higher dose. Twenty-seven patients received only one dose; the maximum number of courses given to one patient was five. Table 3 summarizes the evaluation of response. After 41 courses (66%), there was objective evidence of disease progression at 6 weeks, with six of the patients having died of their disease. Objective responses were noted in two patients. A 67-year-old woman with a well-differentiated rectal primary tumor showed a diminution in the size of her liver metastases after a second course of chlorozotocin, but this lasted only 6 weeks. The second response occurred in a 57-year-old man with a moderately differentiated rectal carcinoma who received the higher dose. There was a diminution in his solitary pulmonary metastasis after the initial course of treatment. This response lasted 20 weeks before objective disease progression occurred. A 40-year-old man with a moderately differentiated rectal carcinoma experienced subjective improvement in pain from hepatic metastases after two courses of treatment, although a liver scan showed no objective evidence of regression.

Overall survival for the group ranged from 2 to 90 weeks, with a median of 19 weeks. The two re-

TABLE 3.—Evaluation of response

Response type	No. of responses
Regression	2 (3%)
Stable disease	19 (31%)
Disease progression	41 (66%)

TABLE 4.—Symptomatic toxicity

	Toxic effect			
	Nausea	Vomiting	Diarrhea	Stomatitis
No. of patients	24	20	5	3
No. of courses	27	21	5	3

sponders survived 49 and 58 weeks, respectively, and the symptomatic responder survived 33 weeks.

Symptomatic toxicity (table 4) was confined to gastrointestinal problems. Twenty-four patients (62%) experienced nausea after a total of 27 courses, and 20 patients experienced vomiting after a total of 21 courses. Five patients had diarrhea. In all cases the symptoms were short-lived and controllable with simple medication.

Hematologic toxicity (wbc count < 3500/mm^3 and platelet count < 110,000/mm^3) was not noted in any of the patients, although 3 weeks after injection, one patient receiving the higher dose had a platelet count nadir of 110,000/mm^3 which recovered spontaneously. There was no biochemical evidence of renal toxicity from the drug nor any acute elevations of liver enzymes.

DISCUSSION

Objective response rates to chemotherapeutic agents in advanced colorectal cancer are poor. Studies using the nitrosoureas alone or in combination in advanced colorectal cancer have shown response rates varying from 10% to 25% (5–7). Chlorozotocin, with the theoretic advantage of an improved therapeutic index, might be expected to be a more effective drug.

This study, using an initial dose of 120 mg/m^2, produced objective responses in two of the 39 patients in the study, although one patient had subjective improvement after each of two injections. This represents an objective response rate of 5% and was achieved at the expense of mild gastrointestinal toxicity in 62% of patients after 44% of the courses. It is noted that both objective responses and the symptomatic response occurred in patients with rectal primary tumors. Without a comparative study, comments on survival times are limited, although it should be noted that both responders had a survival time in excess of twice the median value. The absence of hematologic toxicity in this study suggests that a higher dose of chlorozotocin could have been used and might have been accompanied by a greater tumor response. Alternatively, the low hematologic toxicity of chlorozotocin would allow its use in combination chemotherapy.

From this study it can be concluded that, at a dose of 120 mg/m^2, chlorozotocin has minimal objective evidence of activity against advanced colorectal cancer. However, this might be the result of insufficient dosage rather than tumor insensitivity and would suggest further studies using a higher dose schedule.

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Phase I–II Trial of Methyl-GAG in the Treatment of Patients With Metastatic Renal Adenocarcinoma[1,2]

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SUMMARY

Methyl-GAG, a polyamine synthesis inhibitor, was prospectively evaluated in the treatment of advanced renal adenocarcinoma. Twenty-five patients with measurable disease received methyl-GAG weekly at a starting dose of 500 mg/m² iv, with dose escalation by 50 mg/m²/week (maximum dose, 825). All 25 patients are evaluable for response. Four of these patients (16%) achieved responses including three partial responses and one complete response, with a median duration of 9 weeks (range, 4–15). Nine patients (36%) remained stable and 12 (48%) had progressive disease. In the four responders, regression of disease occurred within the first 4 weeks of therapy. Toxic effects were generally mild and included nausea or vomiting (68%), myalgia (44%), mucositis (40%), neuralgia (40%), weight loss (32%), diarrhea (24%), skin rash (8%), leukopenia (8%), and genital ulcers (4%). We conclude that methyl-GAG has clear, albeit limited, activity against renal adenocarcinoma.

[Cancer Treat Rep 65:17–20, 1981]

Methyl-GAG is a substituted guanylhydrazone which was synthesized in 1958 and found to have significant antineoplastic activity in murine tumor systems (1,2). Its mechanism of antitumor effect has not been elucidated, but in vitro studies have shown methyl-GAG to be a potent inhibitor of *S*-adenosyl-L-methionine decarboxylase, which blocks the synthesis of the polyamine compounds, spermine and spermidine, which, in turn, play a role in DNA and RNA metabolism (3–5).

Early clinical trials using daily iv administration of methyl-GAG demonstrated significant activity against acute nonlymphocytic leukemia (6,7), but at the price of marked (often prohibitive) toxic effects which included mucositis, colitis, dermatitis, myelosuppression, and hypoglycemia (6,7). Because of this severe toxicity, and with the introduction of newer antineoplastic agents, interest in methyl-GAG faded. Recently, however, the results of phase I–II trials conducted by several cooperative groups have suggested antitumor activity against a variety of tumors, using dose-schedule regimens producing more tolerable toxicity (8,9).

In the Southwest Oncology Group study, three of four patients with metastatic renal adenocarcinoma showed improvement (including one partial responder) during treatment with methyl-GAG (9). Because conventional chemotherapy for this disease has proven unsatisfactory, with few studies re-

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porting response rates > 20%–30% (10–16), we have prospectively evaluated the efficacy of methyl-GAG in 25 patients with advanced renal adenocarcinoma.

MATERIALS AND METHODS

Patient Profile

Between June 1979 and January 1980, 25 patients were entered in this study (*see* table 1). All patients had histologic confirmation of advanced renal adenocarcinoma with objectively measurable metastatic disease. All patients were ambulatory, with a median performance status of 0.5 (range, 0–4, Eastern Cooperative Oncology Group [ECOG] scale); they had an estimated survival > 2 months and had normal parameters of hematologic and renal functions (creatinine clearance ≥ 30 ml/min/m²). The median age was 53 years (range, 13–68); there were 19 males and six females. Thirteen of the patients had received prior systemic therapy (chemotherapy, hormonal therapy, or both) for their metastatic disease. Predominant sites of measurable disease in the 25 patients included lung (18), liver (five), soft tissues (one), and lymph nodes (two). Written informed consent was obtained from all patients prior to treatment.

Treatment Plan

Methyl-GAG is formulated in 30-ml vials, each containing 1.0 g. The drug is reconstituted in sodium chloride, 0.9% (USP) at a final concentration of 2.5 mg/ml, with the proper dose administered as a 45-minute iv infusion. Based on the favorable experience of Knight et al (9), methyl-GAG was given as a weekly injection to outpatients at a starting dose of 500 mg/m². Doses were escalated by 50 mg/m²/week until toxic effects failed to resolve by the time of the next injection. On such occasions, drug dose was kept constant or was temporarily de-escalated by 10% (50 mg/m²), 50%, or 100%, depending on the severity of toxicity. Thereafter, the dose was generally held at the maximum tolerable level. The highest dose given to any patient was 825 mg/m².

TABLE 1.—Patient characteristics in phase I–II trial of methyl-GAG in the treatment of advanced renal adenocarcinoma

Characteristic	
Total No. of patients entered	25
Males/females	19/6
Median age in yrs (range)	53 (13–68)
Median performance status* (range)	0.5 (0–4)
No. of patients with prior therapy for metastatic disease	13
Chemotherapy alone	3
Hormonal therapy alone	5
Chemotherapy and hormonal therapy	5
No. of predominant sites of measurable disease† in —	
Lung	18
Liver	5
Soft tissues	1
Lymph nodes	2

*ECOG scale.
†1 patient had 2 sites.

Study Parameters

Prior to treatment, all patients underwent routine history and physical examination with biochemical and radiologic documentation of extent of disease. Initial laboratory studies included cbc, BUN, creatinine, glucose, transaminases, lactate dehydrogenase, alkaline phosphatase, calcium, total protein, albumin, 24-hour urinary creatinine clearance, and chest roentgenogram. Physical examination and cbcs were performed prior to each dose of therapy. Pertinent studies documenting extent of disease were repeated at approximately 4-week intervals.

All patients receiving a minimum of three weekly doses of methyl-GAG were evaluable for response. Response criteria were defined as: *complete response*—the disappearance of all evidence of disease for ≥ 4 weeks; *partial response*—a > 50% decrease in the product of the longest perpendicular diameters of the most measurable areas of tumor for ≥ 4 weeks; *stable disease*—no significant change in measurable disease (minor disease regression failing to meet the requirements of partial response are included in this category); and *progressive disease*—a > 25% increase in the product of tumor perpendicular diameters or the development of new lesions.

Treatment of individual patients generally was continued until clear evidence of disease progression was determined or until the patient requested that therapy be discontinued.

RESULTS

Therapeutic Responses

Table 2 shows that 25 patients received a total of 198 doses of methyl-GAG, with a median of five doses and a range of three to 22 (one dose constitutes a single weekly iv injection as indicated in the

TABLE 2.—Therapeutic responses in 25 patients with advanced renal adenocarcinoma treated with methyl-GAG

No. of doses given	198
Median	5
Range	3–22
No. of patients evaluable for response	25
Complete*	1 (4%)†
Partial*	3 (12%)‡
Stable disease	9 (36%)
Progressive disease	12 (48%)

*Overall response rate, 16%.
†Response duration, 15 wks.
‡Response durations: 4, 5, and 13 wks.

TABLE 3.—Toxic effects in 25 patients with advanced renal adenocarcinoma treated with methyl-GAG

Toxic effect	No. of patients
Nausea or vomiting	17 (68%)
Myalgia	11 (44%)
Mucositis	10 (40%)
Neuralgia	10 (40%)
Weight loss	8 (32%)
Diarrhea	6 (24%)
Skin rash	2 (8%)
Leukopenia	2 (8%)
Genital ulcers	1 (4%)

Materials and Methods section). Four of the 25 evaluable patients (16%) achieved responses, including three partial responses and one complete response, with a median response duration of 9 weeks (range, 4–15). Nine patients (36%) remained stable and 12 (48%) had progressive disease. In the four responders, regression of measurable disease (pulmonary nodules in two, soft tissue mass in one, and mediastinal lymph nodes and liver nodules in one) was prompt and occurred in the initial 4 weeks of treatment. Two of the four responders had had prior therapy for their metastatic disease (progesterone in one and a combination of doxorubicin, CCNU, vinblastine, and vincristine in the other).

It should be noted that with an evaluability requirement of three weekly doses of methyl-GAG, 14 patients had therapy discontinued within 6 weeks: nine patients developed clear signs of disease progression; four patients, whose measurable disease remained unchanged, experienced unacceptable toxic effects (patient refusal) or a more pressing need of palliative radiation therapy; and one partial responder stopped therapy to undergo surgical excision of his remaining measurable disease. Sixteen patients (including two of four responders) tolerated dose escalations > 500 mg/m^2 (75 of 198 total doses > 500 mg/m^2), while six (including three responders) required dose reduction because of toxicity (28 of 198 total doses < 500 mg/m^2). In view of the relatively small number of responding patients and the difficulty of interpreting whether stable disease reflects drug effect or natural disease history, little can be inferred from this study regarding the relationship between cumulative dose of methyl-GAG given and likelihood of response. However, all four responding patients experienced disease regression within the first 3–4 weeks of treatment and at doses approximating 500 mg/m^2.

Toxicity

Table 3 shows the toxic effects for the 25 patients participating in this study. Major toxic effects included intermittent nausea or vomiting in 17 patients (68%), myalgia in 11 (44%), mucositis in ten (40%), neuralgia (paresthesias) in ten (40%), body weight loss of 10% in eight (32%), diarrhea in six (24%), skin rash in two (8%), and genital ulcers in one (4%). Transient mild leukopenia (wbcs, 1.2–2.4 × 10^3/mm^3, resolving within 1 week) occurred in only two patients. One patient developed transient asymptomatic elevations in liver function tests. Two patients refused additional treatment because of severe toxicity after receiving three weekly doses. In general, however, toxicity was mild, with the majority of patients tolerating weekly doses ≥ 500 mg/m^2. There were no drug-related deaths.

DISCUSSION

Methyl-GAG is a polyamine synthesis inhibitor, which, in early clinical trials, displayed significant antitumor activity, particularly in hematologic malignancies (6,7). However, when given as daily bolus infusions in the range of 150 mg/m^2, it produced severe toxicity, thus diminishing its clinical usefulness. Interest in methyl-GAG has been revived by promising results in several broad phase I–II trials in which the drug was administered in less toxic, intermittent im or iv regimens (8,9). Most notably, the Southwest Oncology Group reported complete or partial responses in patients with transitional cell, colon, esophageal, and renal carcinomas treated with weekly iv injections of 500–700 mg/m^2; toxicity was mild (9). These optimistic results provided the impetus for our phase II study of the efficacy of methyl-GAG in the treatment of advanced renal adenocarcinoma.

Twenty-five patients with histologically documented, measurable, metastatic disease received a total of 198 doses of methyl-GAG given as a weekly bolus infusion starting at 500 mg/m². Four patients demonstrated significant disease regression, for an overall response rate of 16%. While it is therefore evident that methyl-GAG, as a single agent, displays a limited degree of activity in this neoplasm, it does not substantially exceed that of conventional agents such as CCNU (14), hydroxyurea (15), vinblastine (13), or progestin (16). Of potential significance is the fact that, when given as a weekly bolus infusion in the range of 500–700 mg/m², methyl-GAG toxicity is reasonably mild, with significant leukopenia being a rare complication even in patients receiving 15 consecutive doses. It might therefore be practical to combine methyl-GAG with another agent whose major dose-limiting toxicity is myelosuppression. Such a combination might produce additive antitumor activity without overlapping toxicity. To investigate this possibility, clinical trials should be undertaken.

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Comparison of Doxorubicin With Cycloleucine in the Treatment of Sarcomas[1,2]

Edwin D. Savlov,* John M. MacIntyre, Edward Knight, and Janet Wolter[2]

SUMMARY

In this patient series, doxorubicin and cycloleucine at a dose of 300 mg/kg both show response rates in the treatment of advanced soft tissue sarcomas of about 15%. Lower doses of cycloleucine (200 mg/kg) yielded less toxicity but were less effective against the sarcomas (6% response rate, three of 51 patients). There were no complete responses with cycloleucine and there were three with doxorubicin. Survival times for patients receiving doxorubicin were significantly longer than those of patients receiving cycloleucine at doses of 300 mg/kg ($P < 0.001$) or 200 mg/kg ($P = 0.02$). The estimated survival times were 29 weeks for doxorubicin and 21 (300 mg/kg) and 18 (200 mg/kg) weeks for cycloleucine. Toxic effects due to cycloleucine were excessive, with severe thrombocytopenia and central nervous system depression being the most prominent.

[Cancer Treat Rep 65:21–27, 1981]

An earlier report indicated that cycloleucine might be active in the control of leiomyosarcomas. After treatment with 300 mg/kg/day of cycloleucine, six of 13 evaluable patients with advanced disease reported by Aust et al (1) had a ≥ 50% reduction in the size of measurable lesions. In addition, there was one apparent complete remission of tumor. Four of the six patients had remissions lasting 3–24 months. All patients who showed evidence of response had toxic effects including vertigo, slurring of speech, ataxia, or disorientation.

At lower dose levels (40–80 mg/kg/day), cycloleucine has had little objective tumor effect. Bone pain seemed to be diminished in patients with multiple myeloma at the lower dose levels, although no evi-

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dence of objective response could be determined (2). Scattered reports of remissions of multiple myeloma at higher dose levels have been made, although no definitive study has been done.

The efficacy of doxorubicin used alone and in combination (3) for sarcomas has been demonstrated. When used alone, a partial response rate of about 30% has been reported. Major toxic effects have been myelosuppression, nausea and vomiting, alopecia, and cardiomyopathy appearing after cumulative doses of 500–600 mg/m^2.

The present study, comparing cycloleucine with doxorubicin used alone in patients with a variety of advanced sarcomas, was undertaken by the Eastern Cooperative Oncology Group (ECOG) in early 1972.

MATERIALS AND METHODS

The patients studied included adults who had biopsy-proven soft tissue sarcomas, with measurable lesions, which were beyond standard therapy. These sarcomas included osteogenic and Ewing's but not reticulum cell sarcoma or lymphosarcoma. To be admitted to the study, the patients were required to have progressive, inoperable, regional, or metastatic malignancy, wbc and platelet counts stable in the normal range, measurable disease, expected survival of at least 2 months, and no form of treatment in the 4 weeks prior to entering the study.

Patients with angina pectoris, cardiac pacemakers, frequent premature ventricular contractions, heart blocks, or other significant ECG or clinical signs of myocardial disease were not eligible. Patients were excluded who had a BUN > 20 mg/100 ml, a serum bilirubin > 2 mg/100 ml, or other signs of significant renal or hepatic damage.

Response to therapy was defined as follows: *partial response*—a decrease by at least 50% of the sum of the cross-sectional area of measured masses calculated from the longest diameter times the longest measurement at right angles to the first; and *complete response*—the disappearance of all measured lesions accompanied by subjective improvement.

Doses

Doxorubicin at a dose of 20 mg/m^2 was given iv daily for 3 days every 3 weeks and was later modified to a total dose of 60 mg/m^2 on Day 1 every 3 weeks.

Cycloleucine (300 mg/kg/day) was given iv for 8 days and was repeated after a 4-week gap between the last dose of the preceding course and the first dose of the next. After significant neurotoxicity was noted, this dose was reduced (February 1973) to 200 mg/kg/day iv for 8 days, with a 4-week gap between courses.

To avoid the difficulties arising from possible changes in prognosis for survival, response, and toxicity, patients treated with high-dose cycloleucine are compared with patients treated with doxorubicin who were entered in the study at the same time (ie, before February 1973), and patients treated with low-dose cycloleucine are compared with patients treated with doxorubicin who were entered in the study after February 1973, in assessing the statistical significance of treatment differences.

Crossover to the alternate drug was mandated if disease progressed or if relapse occurred after response.

Patients were randomized at a central operations office after stratification by origin of sarcoma: (*a*) fibrous tissue; (*b*) fatty tissue; (*c*) muscle tissue; (*d*) vascular; or (*e*) other.

Two hundred and eight of 256 patients who were entered in the trial are evaluable with followup available. Forty-eight patients were excluded for the following reasons: not eligible (eight patients), not randomized (23), other protocol violations (15), and followup data not available (two).

Analysis of the association between survival and several on-study characteristics plus treatment was conducted using Cox's proportional hazards model (4) to simultaneously assess the prognostic value of these characteristics plus treatment with respect to survival.

RESULTS

Induction

Table 1 shows the breakdown by tumor origin of the patients included in the study, with objective responses to the treatments. Overall response rates to high-dose cycloleucine and doxorubicin were similar, but the toxicity of the cycloleucine necessitated dose reduction to a much less effective level. The high response rate in leiomyosarcoma reported previously was not confirmed in this study. Activity in leiomyosarcoma is confirmed, even at the lower dose level.

All but three responses were partial. Three complete responses were observed in patients receiving doxorubicin therapy: one case each of synovial cell sarcoma, chondrosarcoma, and leiomyosarcoma. The median start of response, if it occurred, was 28 days after doxorubicin was started and 30 days after cycloleucine was started.

Remission durations were longer in the doxorubicin group. All but one response lasted > 4 weeks. In the cycloleucine group, six of the ten remissions

TABLE 1.—Objective response by treatment and origin of sarcoma*

Origin	Doxorubicin	Cycloleucine (300 mg/kg)	Cycloleucine (200 mg/kg)	Totals
Fibrous tissue	2/14	1/7	0/11	3/32
Fat tissue	1/8	0/1	0/6	1/15
Rhabdomyosarcoma including embryonal rhabdomyosarcoma	1/6	0/4	1/4	2/14
Leiomyosarcoma	3/28	1/9	2/12	6/49
Other muscle tissue	0/1	0/2	0/0	0/3
Angiosarcoma, lymphangiosarcoma, hemangiosarcoma	0/2	1/3	0/1	1/6
Kaposi's sarcoma	1/2	0/0	0/0	1/2
Other vascular	0/2	0/0	0/1	0/3
Osteogenic sarcoma	1/12	1/3	0/4	2/19
Ewing's sarcoma	0/3	0/2	0/0	0/5
Chondrosarcoma	1/3	1/2	0/2	2/7
Mesothelioma	1/5	2/6	0/1	3/12
Other	5/20	0/12	0/9	5/41
Totals	16/106	7/51	3/51	26/208

*Values = No. of responses/No. of patients treated.

lasted 4 weeks, four of seven at the higher dose (300 mg/kg). Median response durations were 36 weeks for doxorubicin responders and 4 weeks for high-dose cycloleucine responders. The response durations of the three low-dose cycloleucine responders were 3, 6, and 14 weeks.

Seven of the 106 doxorubicin-treated patients received a dose of 60 mg/m² on the Day-1 schedule, with one partial response. No noteworthy differences in response, survival, or toxicity were observed among patients receiving this schedule, and they are not analyzed separately in the following analysis.

Patients receiving doxorubicin induction therapy continued to receive therapy longer (median, 62 days) than the patients receiving cycloleucine at either dose level (median, 28 days) ($P < 0.001$ for both). This difference is also reflected in the number of complete or partial courses of therapy given. Only 20 patients (39%) who received 300 mg/kg of cycloleucine and 15 patients (29%) who received 200 mg/kg of cycloleucine were given two or more courses, while all but 14 patients (13%) receiving doxorubicin were given at least two courses. No patient received more than six courses of cycloleucine therapy.

The median number of doxorubicin courses given was four. Eleven patients (10%) in the doxorubicin group received ≥ 90% of the protocol-prescribed maximum dose of 500 mg/m².

Toxicity

Occurrences of drug-related toxicity with doxorubicin are shown in table 2. The most frequent forms of toxicity were hematologic toxic effects, nausea and vomiting, and alopecia. The severe toxic effects listed as "other" include one case of severe fatigue and one case of cardiac toxicity, characterized by frequent atrial premature contractions, dropped beats, first-degree atrioventricular block, and occasional nodal rhythm. There were no instances of life-threatening toxicity.

Severe doxorubicin toxicity occurred principally during the first month of treatment.

Toxicity with cycloleucine (300 mg/kg) is shown in table 3; neurologic toxic effects and nausea and vomiting were the most frequent. Hematologic toxicity was relatively rare; however, when it was present, it tended to be quite severe (three of the ten cases of mild or worse hematologic toxicity were considered to be life-threatening).

The six instances of life-threatening or lethal toxicity were as follows:

a. One patient developed lethal CNS toxic reactions starting as lethargy and confusion on Day 11 of the first course. Because of an earlier incident of CNS toxicity from which she had recovered, the patient was being treated at a two-thirds dose level at the time when the second incident began. The patient died on Day 16.

b. Three patients experienced life-threatening thrombocytopenia: platelet count nadirs were 8000, 1200, and 3000/mm³ on Days 10, 16, and 16, respectively, of the first course. In the first two patients severe leukopenia occurred at about the same time. In all three patients the blood cell counts subsequently recovered.

TABLE 2.—Toxicity with doxorubicin as induction step

Toxic effect	No. of patients*			
	Degree of toxicity			
	None	Mild to moderate	Severe	Life-threatening and/or lethal
Nausea and/or vomiting	54 (51%)	45 (42%)	7 (7%)	0
Diarrhea	103 (97%)	2 (2%)	1 (1%)	0
Stomatitis	88 (83%)	10 (9%)	8 (8%)	0
Other GI	99 (93%)	4 (4%)	3 (3%)	0
Neurologic	104 (98%)	2 (2%)	0	0
Hematologic	47 (44%)	53 (50%)	6 (6%)	0
Other†	93 (88%)	9 (8%)	4 (4%)	0

*Values in parentheses = % of evaluable patients in category.
†Excluding alopecia.

TABLE 3.—Toxicity with 300 mg/kg of cycloleucine as induction step

Toxic effect	No. of patients*				
	Degree of toxicity				
	None	Mild to moderate	Severe	Life-threatening	Lethal
Nausea and/or vomiting	29 (57%)	16 (31%)	5 (10%)	1 (2%)	0
Diarrhea	49 (96%)	0	2 (4%)	0	0
Other GI	48 (94%)	3 (6%)	0	0	0
Neurologic	15 (29%)	20 (39%)	14 (28%)	1 (2%)	1 (2%)
Hematologic	41 (80%)	5 (10%)	2 (4%)	3 (6%)	0
Other	44 (86%)	6 (12%)	1 (2%)	0	0

*Values in parentheses = % of evaluable patients in category.

c. One patient developed life-threatening nausea and vomiting coupled with severe dizziness during the first course. These conditions persisted for 1 week following the last treatment dose.

d. One patient experienced life-threatening CNS toxicity beginning as disorientation on Day 6 and progressing to coma. The patient also experienced life-threatening dyspnea due to disease and died on Day 8.

CNS toxicity typically was most severe during the first 2 days of treatment. Severe toxicity was relatively infrequent after the first month (even after adjusting for the lack of patients continuing beyond the first month of cycloleucine). In particular, there was no life-threatening or lethal toxicity after the first month.

The patterns of toxicity for low- and high-dose cycloleucine were similar, although no life-threatening or lethal CNS toxicity was observed with low-dose cycloleucine (*see* table 4). CNS toxic effects and nausea and vomiting were again the most common severe toxic effects, with hematologic toxicity infrequent but often severe when it occurred. Severe CNS toxic effects and severe nausea and vomiting were slightly less frequent with low-dose than with high-dose cycloleucine. Life-threatening toxicity with low-dose cycloleucine consisted of two cases of thrombocytopenia: platelet count nadirs were 15,000/mm^3 on Day 12 of the first course and 20,000/mm^3 on Day 21 of the first course. Both patients eventually recovered. However, in the first patient, the platelet count did not rise for 2 weeks and took 6 weeks to reach 100,000/mm^3.

Survival by treatment is shown in figure 1. Survival times were significantly longer for the doxorubicin-treated patients than for the high-dose cycloleucine-treated patients ($P < 0.001$) or for the low-dose cycloleucine-treated patients ($P = 0.02$). Median survival times of induction therapy were 29 weeks for doxorubicin-treated patients and 21 (300 mg/kg) and 18 (200 mg/kg) weeks for cycloleucine-treated patients.

Patients with an initial performance status (PS) of 0 (asymptomatic and fully ambulatory) survived significantly longer than patients with an initial PS

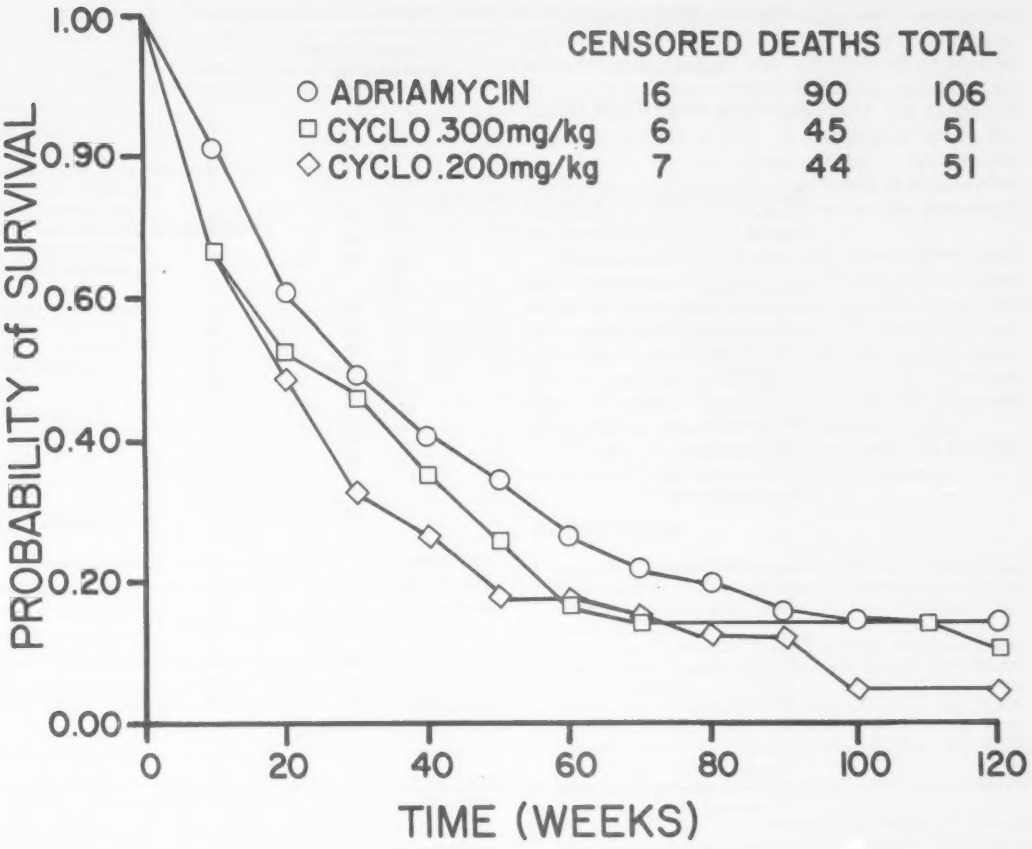

FIGURE 1.—Survival by treatment induction step.

TABLE 4.—Toxicity with 200 mg/kg of cycloleucine as induction step

Toxic effect	No. of patients*			
	Degree of toxicity			
	None	Mild to moderate	Severe	Life-threatening
Nausea and/or vomiting	32 (63%)	17 (33%)	2 (4%)	0
Diarrhea	50 (98%)	0	1 (2%)	0
Other GI	46 (90%)	5 (10%)	0	0
Neurologic	27 (53%)	13 (25%)	11 (22%)	0
Hematologic	42 (82%)	6 (12%)	1 (2%)	2 (4%)
Other	45 (88%)	4 (8%)	2 (4%)	0

*Values in parentheses = % of evaluable patients in category.

TABLE 5.—Median survival time by patient characteristics: induction step

Characteristic	Estimated median survival time (wks)	No. of patients
Ambulatory status—ECOG PS*		
0	57	45
1	31	62
2–4	14	93
Time from 1st symptoms to entry in study (mos)		
0–18	18	122
18–36	34	37
>36	49	49
Tumor origin		
Fibrous tissue	20	32
Fatty tissue	38	15
Muscle tissue	25	66
Vascular	66	11
Other	21	84
Prior surgery		
No	20	64
Yes	27	144
Prior radiotherapy		
No	26	112
Yes	24	96
Prior chemotherapy		
No	26	157
Yes	21	51
Age* in yrs		
≤45	26	73
46–60	33	70
>60	18	64
Sex		
Males	20	102
Females	29	106

*Unknowns excluded.

of 1 (ambulatory but with symptoms) ($P < 0.01$), who in turn had longer survival than nonambulatory patients ($P < 0.001$).

Patients whose time from first symptoms to entry in the study was > 36 months survived significantly longer than those with symptoms between 18 and 36 months ($P < 0.05$). The latter group had significantly ($P < 0.01$) longer survival times than those with symptoms and disease < 18 months. Patients with sarcomas originating in fibrous tissue had significantly shorter survival times ($P < 0.05$ for each comparison) than patients in the other cell type groups, with the exception of the muscle tissue sarcoma group.

Patients without prior chemotherapy had longer survival times than patients with prior chemotherapy ($P < 0.05$).

Table 5 shows the estimated median survival times for these and other patient characteristics.

Crossover

Among patients crossed-over to the alternate drug after failure with the induction drug, there were eight partial responses among the 71 patients given doxorubicin and no responses among the 37 patients given cycloleucine (high-dose cycloleucine, ten patients; low-dose cycloleucine, 27 patients). Median duration of response with crossover was 16 weeks. All but one of the partial responses was accompanied by subjective improvement.

Patients who responded to cycloleucine during the induction step were more likely to respond during the crossover step than nonresponders ($P < 0.05$). Three of the seven patients with crossover therapy who responded to cycloleucine induction therapy also responded to doxorubicin in the crossover step. Table 6 shows response by cell type for patients receiving doxorubicin as crossover treatment.

TABLE 6.—Response to doxorubicin crossover

Origin	No. responding/No. of patients treated
Fibrous tissue	1/12
Fat tissue	1/5
Rhabdomyosarcoma (including embryonal rhabdomyosarcoma)	2/6
Leiomyosarcoma	1/13
Other muscle tissue	0/1
Angiosarcoma, lymphangiosarcoma, hemangiosarcoma	1/3
Kaposi's sarcoma	0/0
Other vascular	0/1
Osteogenic sarcoma	0/7
Ewing's sarcoma	1/1
Chondrosarcoma	0/3
Mesothelioma	1/5
Other	0/14
Total	8/71

DISCUSSION

Response rates for doxorubicin in this series are considerably lower than those reported previously (3). However, the results are consistent with those of Rosenbaum and Schoenfeld (5), who suggested that response of sarcomas to doxorubicin alone or in combination was related to the dose of doxorubicin. They observed a 28% response rate (complete or partial) when a dose of 70 mg/m² was given at 3-week intervals while only a 13% response rate was seen when a dose of 50 mg/m² (along with vincristine and cyclophosphamide) was given. In view of these data, the observed response rate of 15% in this group, most of whom had received previous therapies, is not out of line. Other factors, such as the variety of histologic types of varying degrees of drug sensitivity, must also be invoked.

Cycloleucine performed rather well, considering its severe CNS toxicity and the difficulty of continuing therapy with it. The selective effect on leiomyosarcomas was not evident in this series. At the lower drug dose toxicity decreased, but so did the tumor effect (by > 50%, to a negligible level). Because of its low hematologic toxicity, cycloleucine may prove useful at low dose levels in future combinations of drugs. An antidote to the neurologic toxic effects would be helpful.

Pharmacologic studies in mice have shown fairly slow excretion of cycloleucine; the drug accumulates in tissues mostly in the original compound form with little incorporation into protein. In view of its low excretion rate and lack of metabolism, a single large dose at weekly or longer intervals might be less toxic. In mice, absorption of orally administered drug was as good as with ip injection (2,6).

A study of cycloleucine given orally in a single dose every 7–14 days should be considered.

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Cyclophosphamide, Doxorubicin, and Cisplatin (CAP) in the Treatment of Urothelial Malignancy: A Pilot Study of the Southeastern Cancer Study Group[1,2]

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SUMMARY

Thirty-four patients with urothelial malignancy were treated with a chemotherapy regimen of cyclophosphamide, doxorubicin, and cisplatin. The objective response rate was 38% (13 of 34 patients), with three patients achieving a complete remission. Objective responders had a significantly longer median survival time than nonresponders (225 vs 137 days [$P \leq 0.04$]). The median duration of remission was 177 days. Hematologic toxic effects were initially severe, with three episodes of sepsis and one death, but were acceptable after dose modification. This study demonstrates that the regimen of cyclophosphamide, doxorubicin, and cisplatin is effective in metastatic urothelial malignancy. Although response duration was short (25 weeks), responders clearly lived longer than nonresponders.

[Cancer Treat Rep 65:29–32, 1981]

A pilot study utilizing a combination of cyclophosphamide, doxorubicin, and cisplatin (CAP) was undertaken to evaluate the efficacy and toxicity of this regimen in urothelial malignancy. Each of these agents has reported activity as single drugs. Cyclophosphamide has been reported to have a response rate as high as 64% in patients with local and metastatic disease (1) and a more realistic 26% in patients with metastatic disease (2). Doxorubicin, which has had significantly wider use, has a response rate of 16% in a larger series of patients (3). Cisplatin as a single agent has a reported response rate of 33%, increasing to 45% in the subset of patients with no prior chemotherapy (4). There has recently been a series of preliminary studies reporting response rates from 35% to 90% when combinations of these agents were used (5). This report reviews our initial findings with the CAP combination.

METHODS

Patient Selection

Patients with locally recurrent or metastatic urothelial malignancy were entered in this study if they had at least one measurable lesion, a Karnofsky performance status $\geq 40\%$, a bilirubin ≤ 1.2 mg/100 ml, a granulocyte count $> 1500/mm^3$, and no prior parenteral cyclophosphamide, doxorubicin, or cisplatin. Patients without measurable lesion were evaluated for toxicity only. Evaluation for extent of disease included liver scan, bone scan, bone roentgenograms of scan-positive areas, chest roentgenogram, and 24-hour urine collection for creatinine clearance (Ccr). When indicated, bone marrow biopsy, computerized tomography, and other studies were done.

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A total of 43 patients were entered in this study; 36 were evaluable for toxicity and 34 were evaluable for response. Of the seven patients who were excluded from evaluation, one was ineligible, two were lost to followup, and four were inevaluable because of major protocol violation with inadequate drug dosage. The 36 evaluable patients included 32 males and four females ranging in age from 49 to 75 years (median, 65). Seventeen patients had had prior radiation therapy, but none had had prior parenteral chemotherapy. Thirty-five patients had transitional cell carcinoma of the bladder, ureter, or renal pelvis. One patient had squamous cell carcinoma of the ureter. The overwhelming majority of the 36 patients (92%) had at least one or two organ systems involved: lung metastases (nine patients; 25%), liver metastases (11; 31%), extra-pelvic lymph nodes (15; 42%), bone metastases (14; 38%), and local pelvic disease defined as bladder, rectal, vaginal, or perineal recurrence (11; 31%).

Protocol Design

Chemotherapy was given every 21 days for three courses, with evaluation at 9 weeks unless there was rapid disease progression. Patients with stable disease or objective response continued to receive treatment until disease progression. The initial dose of chemotherapy was: 500 mg/m^2 of cyclophosphamide, 50 mg/m^2 of doxorubicin, and 40 mg/m^2 of cisplatin. Patients with Ccr < 75 ml/minute were initially given a one-half dose of cisplatin with dose escalation if no significant elevation of BUN, creatinine, or Ccr resulted. Because of significant myelotoxicity in the initial group of patients treated (with three episodes of sepsis including one death), the doses of cyclophosphamide and doxorubicin were reduced by 20%, to 400 and 40 mg/m^2, respectively. Since renal toxicity appeared minimal in this initial group of patients, the dose of cisplatin was escalated to 50 mg/m^2.

Criteria for response are those of the Southeastern Cancer Study Group (SECSG). A complete response (CR) is defined as the absence of clinically detectable disease, and a partial response (PR) is defined as at least a 50% reduction in the sum of the products of perpendicular diameters of the measurable lesions without new lesions. Stable disease is defined as a ≤ 25% increase in the sum of products of perpendicular diameters of measurable lesions with no new lesions appearing. Progressive disease is defined as not meeting the criteria for CR, PR, or stable disease. Responses were defined from the start of therapy and had to be maintained for at least 4 weeks. Toxicity was likewise graded by standard SECSG criteria (6) with additional modifications as follows. Renal toxicity was graded as an increase of serum creatinine up to 3 mg/100 ml or reduction of Ccr by 25%; this resulted in a reduction of cisplatin dose by 25%. If serum creatinine was > 3 mg/100 ml and/or Ccr reduction was > 50%, cisplatin was not administered until Ccr or serum creatinine returned to pretreatment levels.

Informed consent was obtained from all patients entering the study.

RESULTS

Response to treatment is summarized in table 1; the median survival from the start of treatment and median duration of response are shown in table 2. The overall objective response rate is 38%. Of 34 patients, three (9%) achieved a CR and ten (29%) achieved a PR. An additional six patients (18%) had a stabilization of disease although all had documented disease progression before the start of treatment. Objective responders (CR plus PR) had a statistically significant longer median survival time (MST) than patients with progressive disease. The MST of objective responders was 225 days as compared to 137 days for patients with progressive disease. Although patients with stable disease had a longer MST (179 days) than those with disease progression, the difference was not statistically significant. The response duration of objective responders was 177 days (ie, 25 weeks). The median CR duration was 162 days (range, 112 to 311) and the median PR duration was 167 days (range, 42+ to 204).

Responses to treatment were seen in diverse categories of metastatic disease (table 3), including liver (three of ten patients), local pelvic recurrence (three of 11), extra-pelvic lymph nodes (six of 14), healing of lytic bone disease (three of 14), and pulmonary metastases (two of nine). An analysis of the following possible prognostic factors showed no relationship between response at 9 weeks and variables such as sex, prior radiation therapy, age, performance status, and pattern of metastases at the start of treatment. A review of the median number of courses of chemotherapy revealed that responders and patients with stable disease received significantly more treatments (5.5 and 4.5, respectively) than patients with disease progression (three courses). This was significant at the $P \leq 0.03$ level (Kruskal-Wallis analysis).

Toxicity

Gastrointestinal toxicity was significant, with most patients experiencing nausea and vomiting

TABLE 1.—Response of urothelial malignancy to treatment with CAP

Response	No. of patients (%)
CR	3 (9)
PR	10 (29)
Stable disease	6 (18)
Disease progression	15 (44)
Total	34 (100)

TABLE 2.—Survival and response duration for patients treated with CAP

Patient group	Median survival (days)	Median duration of response (days)
Responders (CR + PR)	225*	177
Stable disease	179	128
Disease progression	137	

*Responders vs patients with progressive disease: $P \leq 0.04$ (generalized Wilcoxon test).

that was not relieved by antiemetics. Some patients were able to tolerate outpatient chemotherapy, and only 9% of the patients required hospitalization for intractable nausea and vomiting. Only one patient entered in the study refused to continue chemotherapy because of severe gastrointestinal toxic effects.

Renal toxicity with these relatively low doses of cisplatin was not a significant problem. Only 41% of the patients had a 25%–50% reduction of Ccr, and none required cessation of therapy. One patient had a > 50% reduction of Ccr but did not require dialysis. No patient experienced ototoxic effects and none developed peripheral neuropathy secondary to cisplatin.

Hematologic toxicity was initially severe and led to dose modification. There were three episodes of sepsis (all patients with prior radiation therapy), and one of the patients died. Forty-seven percent of the patients (including those with sepsis) had granulocyte count nadirs of < 2500/mm³. Thrombocytopenia was much less of a problem, and only two patients (6%) had platelet counts of < 50,000/mm³ with one additional patient having nonfatal hemorrhage secondary to thrombocytopenia. After the doses of cyclophosphamide and doxorubicin were modified, adequate granulocyte count nadirs continued to occur without further episodes of sepsis.

TABLE 3.—Disease presentation and response

Category	No. of patients		
	Total	CR	PR
Pulmonary	9	2	
Hepatic	10	2	1
Skeletal	14		3
Extra-pelvic lymph node	14	4	2
Pelvic	11		3

DISCUSSION

Results of this study indicate that CAP is an active regimen in the treatment of urothelial neoplasia. There was a 38% objective response rate and an 18% stabilization rate. Objective responders lived significantly longer than patients with progressive disease ($P \leq 0.04$, generalized Wilcoxon test). The response duration of 25 weeks is similar to the 5–7 months reported by Yagoda et al (7). Toxic effects were clearly acceptable although there was significant granulocytopenia even with the modified dosage.

The most effective chemotherapeutic regimen for the treatment of metastatic bladder carcinoma remains to be identified, although this report continues to document statistically significant prolongation of survival ($P \leq 0.04$, generalized Wilcoxon test) in responding patients as have others (12). It should serve as a stimulus to continued evaluation of what has been a discouraging tumor to treat in the past. The CAP regimen is clearly active in urothelial malignancy and is currently being utilized as adjuvant chemotherapy for bladder carcinoma in a randomized study initiated by the SECSG following preoperative radiation therapy and radical cystectomy.

Recent studies have centered on cisplatin as one of the more active single agents in bladder carcinoma. Yagoda et al (7) have reported a response rate of 33% with cisplatin alone, which increased to 45% in patients without prior chemotherapy. Although earlier single agent data are less impressive, American and European investigators have found activity of 16% with doxorubicin (3), 20% with 5-FU (R. Smalley, personal communication), and 17% with methotrexate (8). In Egypt, Gad-el-Mawla et al (9) reported a 39% response rate with hexamethylmelamine in patients with transitional cell carcinoma as well as squamous cell carcinoma of the bladder.

Combination chemotherapy has received a number of well-publicized trials, most of which have

included cisplatin. Although initial response rates of 88% with cyclophosphamide, doxorubicin, and cisplatin (ten), and 65% with 5-FU, doxorubicin, and cisplatin (11) have been reported, subsequent analyses by these authors noted a response rate of 41% (12) and 42% (13), respectively. Yagoda et al (7) have not shown a striking increase in remission rate with combination therapy. Response rates of 47% with cisplatin and cyclophosphamide, 50% with cisplatin and doxorubicin, and 57% with cisplatin, doxorubicin, and cyclophosphamide are not markedly different from their previously noted 45% in previously untreated patients given cisplatin alone. Since these studies were sequential and nonrandomized, clearly a controlled randomized study of cisplatin versus a cisplatin-containing combination is of importance to delineate the most effective regimen for achieving a higher CR rate and increased survival. The use of other active single agents such as 5-FU, methotrexate, and hexamethylmelamine must also ultimately be re-examined.

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Doxorubicin, Mitolactol (Dibromodulcitol), and Mitomycin C Treatment for Patients With Metastatic Breast Cancer Previously Treated With Cyclophosphamide, Methotrexate, 5-FU, Vincristine, and Prednisone (CMFVP)[1]

Alfred DiStefano, Hwee-Yong Yap,* and George R. Blumenschein[2]

SUMMARY

Thirty-two patients with metastatic breast cancer refractory to chemotherapy with combinations of cyclophosphamide, methotrexate, 5-FU, vincristine, and prednisone were treated with doxorubicin, mitolactol (dibromodulcitol), and mitomycin C. Two complete and 12 partial remissions were observed for a response rate of 43%. When only those patients who were considered evaluable were included (the 26 patients who had received more than one course of therapy), the response rate was 53%. Hematologic toxicity was moderate-to-severe. Responses were seen in visceral, osseous, and soft tissue tumor sites. The median time to the development of disease progression was 30 weeks for responding patients. The median survival of responders has not yet been reached at 48 weeks.

[Cancer Treat Rep 65:33–38, 1981]

Since the initiation of polychemotherapeutic trials in breast cancer (1), large numbers of patients with metastatic disease have been treated with various combinations of cyclophosphamide, methotrexate, 5-FU, vincristine, and prednisone (CMFVP). Objective response has been reported in 40%–60% of patients treated, with variable durations of response and survival. CMFVP combinations have become the standard chemotherapy regimen for those who are not given doxorubicin in first-line therapy. CMFVP combinations are also being used in large numbers of patients as an adjuvant to surgical treatment of early breast cancer.

Although hormonal manipulations may be useful in patients refractory to CMFVP, most patients will require second-line chemotherapy during the course of their disease. We have examined the usefulness of doxorubicin, mitolactol (dibromodulcitol), and mitomycin C (ADM) in patients who have had prior CMFVP treatment, either for metastatic disease or as an adjuvant to surgery.

MATERIALS AND METHODS

Thirty-two patients with metastatic breast cancer who had been treated with CMFVP combinations were referred to the M. D. Anderson Hospital and Tumor Institute and were entered in the study. All patients had measurable metastatic disease and normal renal, cardiac, and hepatic functions. Response was evaluated by standard criteria (2).

The chemotherapeutic regimen, ADM, consisted of doxorubicin (40 mg/m² of body surface area [BSA], administered iv every 21 days), mitolactol (70 mg/m² of BSA, given orally in one daily dose for 5 days and repeated every 21 days), and mitomycin C (10 mg/m² of BSA, given iv every 42 days). The total dose of doxorubicin was limited to 450 mg/m² of BSA, and the total dose of mitomycin C was 40 mg/m² of BSA. Thereafter, maintenance therapy

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TABLE 1.—Characteristics of 26 evaluable patients treated with ADM

Characteristic	No. of patients (%)
Median age in yrs (range): 55 (35–71)	
Menopausal status	
Premenopausal	3 (11.5)
Postoophorectomy	7 (26.9)
Postmenopausal	16 (61.5)
Median disease-free interval in mos (range): 25.5 (8–84)	
Predominant metastatic site	
Visceral	14 (53.8)
Osseous	7 (26.9)
Soft tissue	5 (19.2)
No. of metastatic sites	
1–2	17 (65.4)
3–4	8 (30.8)
> 4	1 (3.8)

consisted of mitolactol alone at a maximum tolerated dose.

RESULTS

Patient Characteristics

Thirty-two patients were entered in the study. Six patients were inevaluable (three refused further treatment, having demonstrated no evidence of progressive disease after one dose of chemotherapy, two died within 14 days after starting chemotherapy, and one was lost to followup). The characteristics of the 26 evaluable patients are shown in table 1. Most patients had received hormonal therapy and radiotherapy, and all had received various CMFVP combinations for a median of 12 months prior to ADM therapy. In addition to CMFVP combinations, six patients had also received doxorubicin in small, infrequent doses before entering this study;

TABLE 2.—Previous treatment characteristics of evaluable patients treated with ADM

Pretreatment characteristic		
No. of patients with—		
Prior hormonal therapy		
Additive	13	(50.0)*
Ablative	5	(19.2)
Prior radiotherapy		
Postoperative	10	(38.5)
Soft tissue	2	(7.7)
Bone	6	(23.1)
Prior chemotherapy		
CMFVP combinations	18	(69.2)
CMFVP + melphalan	2	(7.7)
CMFVP ± melphalan + doxorubicin	6	(23.1)
Prior doxorubicin dose (mg/m^2 BSA)		
Median	75	
Range	50–235	
Intent of prior chemotherapy (No. of patients)		
Adjuvant to surgery	17	(65.4)
Sole treatment for metastatic disease	5	(19.2)
Both	4	(15.4)
Duration of therapy (mos)		
Median	12.0	
Range	3–25	
Time of disease progression (mos)		
With chemotherapy	17	(65.4)
After chemotherapy	9	(34.6)
Median duration after chemotherapy	16.0	
Range	4–24	

*Nos. in parentheses = %.

TABLE 3.—Response of patients treated with ADM

	No. of patients—		
	Evaluable	Responding	% responding
Total	26	14	53.8
Type of response			
Complete	26	2	7.7
Partial	26	12	46.1
Stable disease	26	5	19.2
Progressive disease	26	7	26.9
Site of response			
Bone ± soft tissue		6	
Soft tissue		4	
Visceral		4*	
Premenopausal postoophorectomy	10	5	50.0
Postmenopausal	16	9	56.3
Sites of metastases (1 or 2)	17	12	70.6
Sites of metastases (≥ 3)	9	2	22.2
Adjuvant CMFVP failures	17	10	58.8
Sole CMFVP ± adjuvant CMFVP failures	9	4	44.4
Progressed with CMFVP	17	9	52.9
Progressed after CMFVP	9	5	55.5
Estrogen receptor-positive	4	2	50.0
Estrogen receptor-negative	6	1	16.7
Estrogen receptor unknown	16	11	68.8
Prior doxorubicin exposure	6	2	33.3

*Includes 2 lung, 1 liver, and 1 pleural effusion.

five of these had received less than three doses of doxorubicin. Seventeen patients had progressive metastatic disease while actively receiving CMFVP chemotherapy, and nine patients developed new metastatic disease, having completed chemotherapy a median of 16.0 months before entering this study. Seventeen patients had failed CMFVP adjuvant treatment, five had failed treatment with CMFVP for metastatic disease, and four others had failed treatment consisting of CMFVP used both as an adjuvant to surgery and for metastatic disease (table 2).

Response

There were 14 responses among 26 evaluable patients (those who received more than one course of therapy), resulting in a response rate of 53.8%. When all 32 patients entered in the study were included, the response rate was 43%. There were two complete and 12 partial responses (≥ 50% reduction in tumor measurements). Only seven patients (27%) had progressive disease during therapy (table 3). Responses were seen predominantly in patients with soft tissue disease (four patients, including both complete remissions), osseous metastases (six), and visceral involvement (four). The response rate was equal in premenopausal and postmenopausal women, as well as in those patients who developed progressive or new disease while receiving CMFVP chemotherapy and in those who developed new metastatic disease when CMFVP chemotherapy was discontinued. Patients with low tumor burdens and those who had primary adjuvant treatment failures responded more often than patients with high tumor burdens and those who had failed treatment of metastatic disease. Estrogen receptor data were available in only ten patients. Two of four estrogen receptor-positive patients responded, while only one of six estrogen receptor-negative patients responded.

The median time to disease progression for responders was 30 weeks. Both responders and those with stable disease had longer times to disease progression than nonresponders (fig 1). The median survival times were 15 weeks for nonresponders

TABLE 4.—Hematologic toxicity in patients treated with ADM

Course No.	Median total wbc count nadir (cells × 10^3/mm^3)	Day of nadir	Median absolute granulocyte count nadir (cells × 10^3/mm^3)	Day of nadir	Median platelet count nadir (cells × 10^3/mm^3)	Day of nadir
1	2.2	12	1.1	12	187	12
2	2.8	15	1.5	14	160	11
3	3.0	14	1.5	14	126	17
4	2.1	16	1.0	14	66	9
5	2.0	14	0.6	17	82	14

and 40 weeks for patients with stable disease; the median survival of responders has not yet been reached (fig 2).

Toxicity

Nonhematologic toxicity was mild. Alopecia was seen in all patients, and nausea and vomiting were observed in 80%. No episodes of congestive heart failure or renal or pulmonary toxicity were noted. Hematologic toxicity was moderately severe (table 4). Cumulative thrombocytopenia was observed, and mitomycin C was prematurely discontinued in three patients (in two patients after total doses of 30 mg/m^2 and in the other after 20 mg/m^2). There were no episodes of bleeding, and platelet recovery

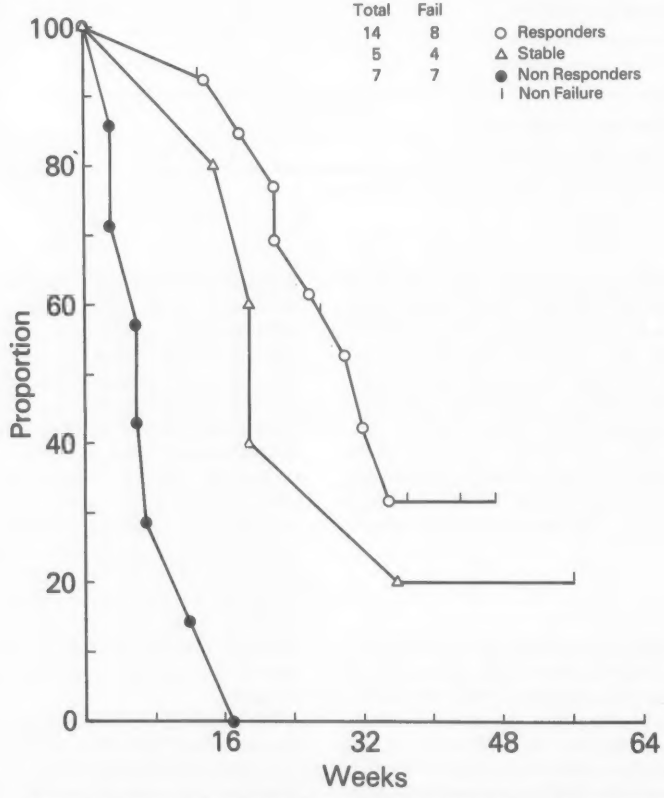

FIGURE 1.—Time to development of progressive metastatic disease in patients receiving ADM.

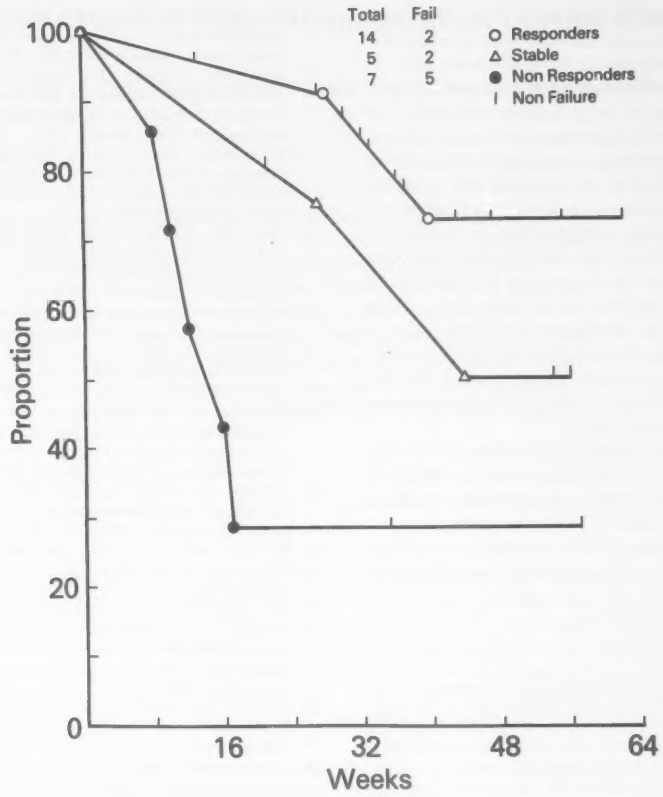

FIGURE 2.—Length of survival of patients receiving ADM.

followed discontinuation of mitomycin C in all patients. Subsequent courses of doxorubicin and mitolactol were well-tolerated. Drug dosage was adjusted to allow a granulocyte count nadir of between 1000 and 1500/mm^3. There were no other documented episodes of infection, except for one episode of septicemia, which contributed to the early death of one of the inevaluable patients.

DISCUSSION

Doxorubicin therapy for patients with metastatic breast cancer who have received prior chemotherapy with CMFVP combinations has been disappointing. Responses have been seen in 21%–33% of the patients and have been of short duration (3–5). Some doxorubicin combinations in this group of patients have not improved the results. Doxorubicin combined with CCNU has produced responses in 26% of patients (6,7); combined with methyl-CCNU (8), it has produced responses in 18%; and combined with vincristine (9), it has produced responses in 22%. Somewhat better results were obtained with doxorubicin, BCNU, and cyclophosphamide (10) (44% response rate); doxorubicin and mitolactol (11) (40% response rate); and doxorubicin and mitomycin C (48% response rate) (12). The combination of doxorubicin, vinblastine, thioTEPA, and Halotestin (13) has given responses in 52% of a small number of patients, producing an 11.5-month remission duration.

Mitomycin C (14) and mitolactol (11,15,16) are active single agents in metastatic breast cancer. Our combination of ADM has produced responses in 43% of all patients entered in the study and in 71% of the patients with low tumor burden. The failure of many treatment regimens in patients who have had prior chemotherapy and the high response rate in

patients with only one or two sites of involvement emphasize the prognostic factors that predict for response to chemotherapy in breast cancer (2).

Only the studies of Tormey et al (11) and Morgan (12), using doxorubicin with either mitolactol or mitomycin C, are directly comparable to our report. In both studies, objective response (complete or partial) was seen in 40% of the patients treated. The relative contribution of mitomycin C or mitolactol to our three-drug combination is uncertain, but the three-drug induction combination appears to produce a response in a greater proportion of patients who have received more than one course of therapy (54%). The favorable response rate in patients who were clearly resistant to CMFVP combinations (the 17 patients who progressed while receiving CMFVP) indicates lack of cross-resistance between CMFVP and ADM.

ADM is an effective combination for the treatment of patients with breast cancer who have shown resistance to CMFVP combinations. This regimen deserves further evaluation, especially for patients who have failed adjuvant chemotherapy. Addition of hormonal agents may improve the usefulness of this regimen.

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Early Pulmonary Toxicity After Administration of High-Dose BCNU[1,2]

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SUMMARY

Patients with solid tumor malignancies who failed extensive front-line treatments were treated with high-dose BCNU followed by autologous bone marrow transplantation. Since 1976, 14 patients were treated in this program with BCNU doses ranging from 600 to 1500 mg/m² over 2 days. Three patients developed pulmonary toxicity only a few weeks after receiving high-dose BCNU at total doses of 900, 1000, and 1500 mg/m². Two of the three patients had had prior irradiation to the primary tumor. The third patient did not have prior irradiation to the chest but had received prior nitrosourea therapy. The use of high-dose BCNU in transplantation may be limited by early pulmonary toxicity.

[Cancer Treat Rep 65:39–44, 1981]

Pulmonary fibrosis with interstitial pneumonitis has been reported as a toxic effect after prolonged administration of several drugs (1,2). These drugs include busulfan (3,4), cyclophosphamide (5), methotrexate (6), and bleomycin (7). In addition, there have been several reports of pulmonary fibrosis resulting from long-term administration of low-dose BCNU (8–13). The lowest total dose of BCNU, when administered without other cytotoxic agents that resulted in pulmonary fibrosis, was 580 mg/m² (total, 1383 mg) given over eight courses for 1 year (7). The other reported cases were at doses of 1550 mg/m² over 26 months (8), 2.08 g over 22 months (9), 1.2 g/m² (total, 2.3 g) over 17 months (10), and 2.08 g/m² (total, 3.3 g) over 14 courses for 35 months (11). However, Durant et al (13) reported nine cases of pulmonary toxicity after the concurrent administration of BCNU and cyclophosphamide. Seven of these patients had total BCNU doses of < 670 mg/m², and one patient experienced toxic effects at a total BCNU dose of 400 mg/m². Except for this one patient, the time to the onset of symptoms has been delayed, occurring between 5 and 43 months.

Since 1976, we have used high-dose BCNU in association with autologous bone marrow transplantation to treat patients with solid tumors who have failed extensive front-line treatment (14,15). We have given BCNU in doses ranging from 600 to 1500 mg/m². In this group of patients, we have observed three patients who developed pulmonary fibrosis only a few weeks after receiving high-dose BCNU. We are reporting here the three patients who developed pulmonary fibrosis a short time after high-dose BCNU was administered.

CASE REPORTS

Patient 1

Patient 1, a 54-year-old male, was referred to M. D. Anderson Hospital and Tumor Institute (MDAH) in November 1976 with limited oat cell carcinoma

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[4]We express our appreciation to Ms. Lee Merriweather for her secretarial assistance.

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of the lung. He was treated with combination chemoimmunotherapy consisting of VP-16-213, ifosfamide, vincristine, doxorubicin, and *Corynebacterium parvum*, and achieved complete remission after 4 months of treatment. He received maintenance treatment with the same drugs for another 14 months. He also received radiation therapy (3000 R) to the primary tumor as well as prophylactic whole-brain irradiation. In July 1978, just 1 month after all treatments were terminated, he developed fever, cough, and moderate shortness of breath. Chest roentgenogram showed a right upper lung tumor and atelectasis as well as pleural thickening. The left lung was clear. Bronchoscopy and washings confirmed the recurrence of tumor. On August 22, 1978, he started receiving cyclophosphamide (1.5 g/m^2 × 3 days), VP-16-213 (150 mg/m^2 × 3 days), and BCNU (300 mg/m^2 × 1 day). Six days later, autologous bone marrow cells, previously collected and stored, were infused. This course was associated with moderate mucositis, perirectal infection, and severe myelosuppression. Hematologic recovery was achieved 35 days from the first day of treatment. Bone marrow was again collected and stored. Although there was some improvement in his respiration, no tumor reduction was noted. On October 4, 1979, a second course of treatment was given, but only with BCNU at a dose of 300 mg/m^2 × 2 days. Autologous bone marrow rescue was given on Day 5 from the first day of chemotherapy. Two weeks later, his breathing became worse, but a lung examination revealed no rales or wheezes. Blood gas studies showed a pH of 7.48, a pCO_2 of 38 mm Hg, and a pO_2 of 60 mm Hg. Chest roentgenogram showed no active inflammatory infiltrate, and he was treated symptomatically with terbutaline sulfate and intermittent positive pressure breathing. A few days later he became dyspneic and tachypneic, and rales were appreciable throughout both lungs. Chest roentgenogram revealed a diffuse alveolar process in the left lower lung, compatible with an inflammatory process. Blood gas studies showed a pH of 7.45, a pCO_2 of 40 mm Hg, and a pO_2 of 40 mm Hg, despite administration of 100% oxygen. He started receiving Bactrim. Culture studies done before antibiotics were started were later found to be negative for bacterial or fungal growth. The following day his condition deteriorated. He became further hypoxic and subsequently died despite intubation.

Autopsy.—The autopsy was limited to the chest. There was remaining viable oat cell carcinoma, measuring 4 × 3.5 × 3.0 cm, in the right upper lobe. This was present in the right hilar lymph node and in multiple small sites in the thoracic bone marrow samples. The right lung weighed 800 g. The remaining parenchyma on this side was subcrepitant, but otherwise not remarkable. There were right (700 ml) and left (500 ml) pleural effusions. The left lung weighed 1200 g, did not show tumor metastases, and was rubbery and red throughout. Post-mortem lung and blood cultures for bacteria and fungi showed no significant growth.

Patient 2

Patient 2, a 39-year-old female, was referred to MDAH on September 20, 1978, with adenocarcinoma of the right lung with paratracheal lymph node and brain metastases. She previously had a right pneumonectomy and irradiation (5000 R) to the mediastinum. She also received whole-brain irradiation. When first seen at MDAH, her physical examination was unremarkable, and no treatment was given. She was followed regularly and during this time had multiple bone marrow aspirations for cryopreservation.

On December 19, 1978, she developed "seizures" described as temporary aphasia with no loss of consciousness. Physical examination was unremarkable and neurologic examination showed no abnormalities. Computerized tomographic scan of the brain demonstrated a lesion within the right cerebellar pontine angle and an area of diminished attenuation in the right temporo-occipital region. On December 30, 1978, she started receiving BCNU at a dose of 500 mg/m^2 for 2 days followed by autologous bone marrow rescue using her stored marrow. In early January 1979, she developed metastatic disease on the right knee, characterized by severe pain. Radiation treatment resulted in relief of pain. On January 24, 1979, 3 weeks after BCNU therapy, she developed severe pain in the area of the left breast, somewhat aggravated by inspiration and relieved by holding her breast. On examination, the area above the left breast seemed swollen but was not red or warm. The left breast was tender to pressure. There was an occasional wheeze with diminished breath sounds over the left lung posteriorly. No rub was appreciable. Chest roentgenogram showed a minimal infiltrate at the left base at the costophrenic sulcus which was not present in the study of January 19, 1979. Blood gas studies showed a pH of 7.41, a pO_2 of 49 mm Hg, and a pCO_2 of 45 mm Hg. She was afebrile and was given oxygen inhalation, which resulted in relief of symptoms. In the first week of February, she developed progressive shortness of breath. Coarse rales were appreciable over the entire left lung and roentgenogram showed increasing areas of consolidation predominantly in the lingular segment of the left upper lobe. She became hypoxic, and her condition deteriorated

rapidly. Despite oxygen inhalation and intubation, she died on February 10, 1979. All cultures for acid-fast bacilli, bacteria, pneumocystosis, cytomegalic virus (CMV), and antibody titers for toxoplasmosis and CMV were negative.

Autopsy.—At autopsy, no tumor remained in the chest, and none was found in the brain, in the originally suspected sites. Metastases were present, however, in both kidneys and in periaortic lymph nodes. The left lung weighed 1080 g and was red and rubbery without localized defects. Post-mortem blood cultures showed *Serratia liquefaciens* and *Citrobacter freundii*. The bone marrow showed a small amount of remaining damage but was 60% cellular, with all three series showing good activity.

Patient 3

Patient 3, a 53-year-old female, had melanoma metastatic to the lungs. She was first seen at MDAH in November 1978, and workup revealed brain metastasis in addition to the pulmonary lesions. Physical and neurologic examinations were normal. She was given radiotherapy to the brain, followed by chemotherapy with PCNU. Definite progression of the brain and lung lesions was noted after the third course. On April 20, 1979, she started receiving BCNU at a dose of 750 mg/m² for 2 days (total, 1.5 g/m²), followed 5 days later by autologous bone marrow transplantation using bone marrow aspirated and stored prior to high-dose BCNU. However, her neurologic condition continued to deteriorate and 2 weeks later she became comatose and subsequently died. A chest roentgenogram prior to her death showed blunting of the right costophrenic angle but no evidence of infiltrate. She showed no signs of pulmonary difficulty prior to her death. Blood gas studies showed a pH of 7.58, a pCO_2 of 30 mm Hg, and a pO_2 of 77.5 mm Hg. Cultures of blood, sputum, and urine, as well as toxoplasma and CMV titers, were negative.

Autopsy.—At autopsy, metastatic melanoma was present in the left lung, brain, one site in the liver, pancreas, and peripancreatic and renal lymph nodes. The right lung weighed 600 g, and the left weighed 400 g. Tracheobronchitis was present, as were multifocal sites of hemorrhage in the right lower lobe. The bone marrow was hypocellular, at 10% cellularity. There was focal damage still evident in the marrow. Blood cultures showed no growth.

SUMMARY OF HISTOPATHOLOGY

The common pathway of diffuse alveolar damage–adult respiratory failure or distress syndrome is well-represented in these three case reports (*see* table 1). Acute hyaline membranes were observed in all patients, and were focally very dense in patient 3 (fig 1). Mild congestion and interstitial edema with round cell infiltrate accompanied this injury. Small vascular thrombi were seen in patients 1 and 2. Alveolar macrophages and alveolar fibrin were prominent in patients 2 and 3. Young organizing fibrosis was evolving in all three patients (fig 2). Pneumonia was beginning in patient 3 and very focally obvious in patient 2. This appeared to be complicating the alveolar damage and organization rather than causing it.

DISCUSSION

BCNU is an alkylating agent which has been used increasingly in clinical research since 1964 for the treatment of various hematologic and solid tumor malignancies. Because of its primarily myelosuppressive toxicity, it was one of the drugs chosen to be used in high-dose cytoreductive therapy in patients whose tumors have failed or ceased to respond to conventional treatment. This drug was given at a high dose, followed by infusion of autologous bone marrow cells previously collected and stored.

The occurrence of pulmonary fibrosis after prolonged treatment with BCNU has been reported by several investigators (8–13). In nearly all cases, the duration of treatment was > 1 year, and the total doses were usually > 1 g/m². The exception was a patient in Durant's series (13) who had Hodgkin's disease and who developed signs and symptoms of pulmonary toxicity only 9 days after receiving BCNU at a dose of 400 mg/m². This patient had received prior irradiation to the mediastinum (4000 R).

Since 1976, we have used BCNU in high doses for the treatment of solid tumor malignancies in association with autologous bone marrow transplantation. BCNU was given in doses ranging from 600 to 1500 mg/m² over 2 days for patients with disseminated malignant melanoma, oat cell carcinoma, and adenocarcinoma of the lung (14). In this total group of 14 patients, we observed three who developed pulmonary toxic effects only a few weeks after receiving high-dose BCNU at total doses of 900, 1000, and 1500 mg/m² over 2 days. One patient had had extensive prior chemotherapy in addition to radiation therapy to the chest for oat cell carcinoma of the lung. The second patient had had no previous chemotherapy although she had received radiation therapy to the mediastinum for adenocarcinoma of the lung. The third patient had had no radiation therapy and had received only a short course of chemotherapy with PCNU, another ni-

TABLE 1.—Histologic characteristics

Patient No.	Hyaline membranes	Interstitial cell	Interstitial edema	Fibrosis	Congestion	Focal hemorrhage	Small thromboemboli	Intra-alveolar fibrin	Intra-alveolar macrophages	Acute pneumonia
1	2+*	1+	Trace	1+	Trace	1+	2+	1+	0	0
2	Trace–2+	1+	Trace	2+	Trace	0	2+	Trace–3+	3+	Trace
3	2–4+	2+	1+	1+	Trace	2+	0	2+	2+	1+

*Degree of pathologic change is scored on a scale of 1–4+.

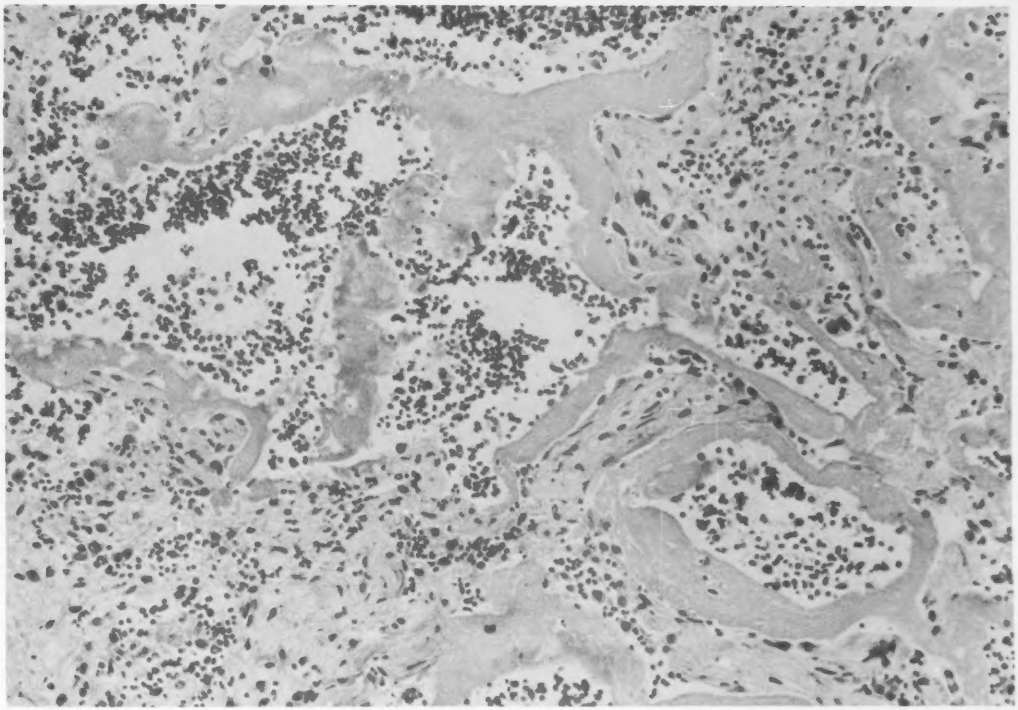

FIGURE 1.—Patient 3: very dense hyaline membranes are seen along with interstitial edema and round cell infiltrate widening alveolar septa (hematoxylin and eosin stain, original magnification × 150).

trosourea, before she received high-dose BCNU (1.5 g/m²). In addition, the latter two patients had received whole-brain irradiation for brain metastasis.

The fact that two of the patients had received irradiation to the mediastinum may suggest that radiation therapy contributed to the development of pulmonary fibrosis, but this does not discount the role of BCNU. The third patient who developed pulmonary fibrosis did not have previous irradiation to the chest, but the anatomic changes at autopsy were even more pronounced than those in the other two patients. What is most important is that BCNU can cause pulmonary toxicity within a short time when given at high doses. Our patients do resemble the single patient of Durant's who received a higher-than-normal single dose of BCNU (400 mg/m²) in combination with cyclophosphamide and who developed diffuse interstitial lung disease within a short time (9 days). Thus, nitrosoureas may have a very limited role in an autologous bone marrow transplantation program because, when given over a short time, they have the ability to cause early pulmonary fibrosis at relatively low total doses. Also, patients with previous chest irradiation or chemotherapy may have an increased risk of this early BCNU pulmonary toxic effect and should be excluded from such therapy.

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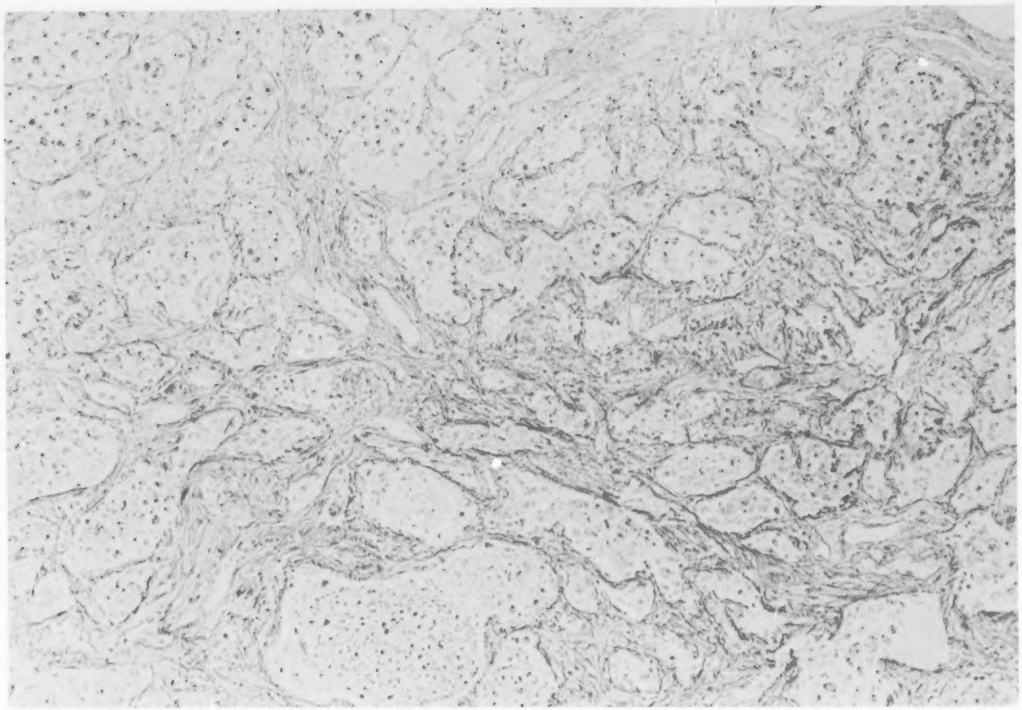

FIGURE 2.—Patient 2: young interstitial fibrosis is shown. Reticulum fibers are widening the interstitium. Compare to the left upper corner where a small portion of more normal lung is seen (Snook Reticulum, original magnification × 60).

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Survival Response of Malignant Gliomas to Radiotherapy With or Without BCNU or Methyl-CCNU Chemotherapy at the University of Kentucky Medical Center[1,2]

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SUMMARY

A total of 115 patients were randomly assigned to one of six therapeutic groups within 3 weeks from craniotomy as part of the Brain Tumor Study Group protocols. In category A (surgery without radiotherapy) there was no significant difference in survival between supportive care only and chemotherapy (BCNU or methyl-CCNU) groups. More than one half of patients in this category died within 6 months of surgery. In category B the addition of 6000 rad of whole-brain radiation treatment to the surgery increased survival time significantly: the 6-month survival rate was two to three times that observed in category A. More than 50% of patients were alive at 1 year and at least 20% survived 2 years. The best survival rate in this study was obtained in patients who received methyl-CCNU with irradiation. The duration of survival was increased to nearly two times that of the radiotherapy group and to six times that of the surgery only group. Reasons for this are discussed.

[Cancer Treat Rep 65:45–51, 1981]

Malignant glioma is the most common malignant brain tumor and invariably follows a uniformly fatal course. Though radiation therapy can make a significant difference in survival, there are no examples of permanent cure in spite of the application of a variety of current treatments. In view of the infiltrative nature of malignant glioma, complete excision of tumor is not possible. The response to radiotherapy and chemotherapy is also limited. The median survival from surgery only ranges from 4.5 to 6 months (1,2) while approximately 20% of patients are alive at 12 months and < 10% at 24 months.

Since the prognosis is not encouraging with surgery (3) with or without radiotherapy, many cooperative groups are studying the value of adjuvant chemotherapy in the treatment of malignant gliomas. We analyzed the influence of chemotherapy on the ultimate course of the malignant gliomas seen in the past 15 years at the University of Kentucky Medical Center. This institution has participated in Brain Tumor Study Group (BTSG) investigations for ~ 15 years, and the present report describes our experience with patient therapy for malignant gliomas using these study protocols. Complete group experiences were reported by Walker and Strike (4). Our experience generally supported conclusions reported by the BTSG but identified a subgroup that responded more favorably than that previously reported (4).

MATERIALS AND METHODS

This study was a controlled, prospective, randomized clinical trial and 115 patients were entered into the protocol program between 1966 and 1977. All patients received traditional neurosurgical eval-

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TABLE 1.—Patient distribution and characteristics

Category	Group	Postsurgical treatment	Age (yrs)		% of patients					No. of chemotherapy courses
					Pathology					
			Mean	Range	Grade III	Grade IV	Biopsy	Partial resection	Total resection	
A	1	None	49	10–70	25	75	41	50	9	—
	2	BCNU	53	33–67	20	80	40	60	—	5
	3	MeCCNU	50	26–63	44	56	44	33	22	4
B	4	Radiotherapy	49	33–67	20	80	44	44	12	—
	5	Radiotherapy + MeCCNU	38	11–61	40	60	36	64	—	6
	6	Radiotherapy + BCNU	47	25–62	31	69	31	50	19	7

uation and treatment. Patients underwent a craniotomy, and those with a confirmed histologic diagnosis of grades III and IV anaplastic glioma, astrocytoma, or glioblastoma were included in this study. We use the term "malignant glioma" because of the variability and differences in diagnostic nomenclature. All histologic diagnoses were confirmed by the study group pathologist.

The protocol consisted of two categories (A and B) and six arms. Within 3 weeks of surgery, patients were randomly assigned by the central BTSG office to one of six designated treatment groups (table 1). The clinical characteristics of patients in each group were analyzed and are presented in table 1. Category A included patients who underwent surgery without postoperative radiotherapy; category B included those who received postoperative radiation treatment (6000 rad to whole brain) following craniotomy. Each category had three arms: no chemotherapy; BCNU at a dose of 80 mg/m²/day for 3 days at intervals of 8 weeks; and methyl-CCNU (MeCCNU) at a dose of 220 mg/m² orally at 8-week intervals starting on the first day of treatment. In category B, during the immediate postoperative period, radiation therapy using cobalt 60 was given to the whole brain. The total tumor dose was 6000 rad in 6 weeks, calculated in the midline with each of two lateral opposed fields treated daily. A compensator was designed at this institution to ensure uniform dose throughout the treatment field (5).

In this study, only survival was used to evaluate the efficacy of treatment modalities since there was no reliable and objective method to assess therapeutic effectiveness. All patients included in this study died during the period of observation, and the survival time was calculated in weeks from the date of surgery. Twenty-eight patients who received no more than two courses of chemotherapy were excluded from evaluation. All others were treated in compliance with the protocol.

RESULTS

The survival of patients assigned to each of six different treatment groups is described below.

Category A (surgery without radiation therapy).— Results are summarized in tables 2 and 3 and figure 1. There were no significant differences in survival between group 1 (supportive care only), group 2 (BCNU only), and group 3 (MeCCNU only); median survival times were 19, 30, and 16 weeks, respectively (table 2). More than one half of patients in category A died within 6 months following surgery. The 1-year survival rate was < 10%, and only one patient survived > 2 years (table 3). Each group had similar percentages of tumors that were either biopsied or resected.

Category B (surgery with radiation therapy).— The addition of radiotherapy to the surgery produced results superior to surgery alone. Thus, the well-established efficacy of radiotherapy was also found here. When radiotherapy was used with adjuvant MeCCNU or BCNU, median survival times were 92 and 69 weeks, respectively (table 2). Groups 4 and 6 were similar with respect to age, tumor grades, and biopsies or resections performed; however, group 5 patients who were treated with MeCCNU plus radiotherapy had a younger median age and a higher percent of grade III tumors.

The differences in survival between category A and B groups were statistically significant (table 2). The 6-month survival rate for the groups that received radiation therapy (category B) was two to three times that for the unirradiated groups. At least 48% of the irradiated patients were still alive at 1 year and roughly one fifth to one third were alive at 2 years (table 3). The median survival time in patients treated with radiation therapy was 47 weeks. This differed from that of patients receiving radiotherapy with adjuvant MeCCNU chemotherapy, which led to a median survival of 92 weeks

TABLE 2.—Median survival in each treatment group

Group	Postsurgical treatment	No. of patients	Median survival (wks)	Significance level*
1	None	12	19	—
2	BCNU	5	30	NS
3	MeCCNU	9	16	NS
4	Radiotherapy	25	47	S
5	Radiotherapy + MeCCNU	10	92	S
6	Radiotherapy + BCNU	26	69	S

*NS = not significant; S = significant at P value 0.05 by Wilcoxon test.

TABLE 3.—Survival rates

Postsurgical chemotherapy	% survival at —*					
	6 mos		12 mos		24 mos	
	No radiotherapy	Radiotherapy	No radiotherapy	Radiotherapy	No radiotherapy	Radiotherapy
None	41.7 (5/12)	72.0 (17/25)	8.3 (1/12)	48.0 (12/25)	8.3 (1/12)	16.0 (4/25)
BCNU	80.0 (4/5)	80.7 (21/26)	40.0 (2/5)	61.5 (16/26)	0 (0/5)	26.9 (7/26)
MeCCNU	33.3 (3/9)	90.0 (9/10)	11.1 (1/9)	70.0 (7/10)	0 (0/9)	30.0 (3/10)

*Nos. in parentheses = No. of patients surviving/No. treated.

(table 2). There was a modest difference for the group receiving radiation therapy combined with BCNU and the group receiving postoperative radiation therapy only. Median survival in the former group was 69 weeks.

At 12 months after surgery, about two thirds of patients (70%) who had been treated with radiotherapy and MeCCNU were alive, whereas slightly less than one half (48%) were alive in the group given postoperative radiation only, and < 10% were alive in the group given neither chemotherapy nor radiotherapy. By 24 months after surgery, three patients (30%) who received radiotherapy and MeCCNU were still alive (table 3).

Distribution of Survival Times

The median survival did not provide a practical parameter in demonstrating differences in response of individual cases though it is a simple parameter for comparing statistical data. Therefore, the distribution of survival times and the mode in survival were studied to get a better insight into survival time after diagnosis and treatment.

The modal survival was determined from the value that appeared most frequently in a grouped frequency distribution. The frequency in the modal interval was computed in percentage to assess the distribution of the modal value grouped in a ± 8-week time interval.

There was a single peak of modal survival in the groups of category A (without radiotherapy) (fig 2). The modal survival in the surgery-only group (group 1) was 16 weeks, with a frequency of 50.0% (table 4). In other words, 50% of patients who received supportive care only survived the postoperative period between 8 and 24 weeks. With BCNU treatment (group 2, sample of only five patients) the modal survival was 32 weeks, with a frequency of 60% (fig 2). With MeCCNU treatment (group 3), modal survival was 16 weeks, with a frequency of 66.7%.

In category B, there appeared to be a wide distribution of survival times (fig 3). The initial failures appeared within 52 weeks in accordance with those of category A groups. However, a second peak beyond the 52 weeks after surgery was observed with a shift of the survival time distribution to the right, indicating increased survival times with radiotherapy.

The first peak in group 4 (radiation therapy only) was at 24 weeks with a frequency of 28%, and the second peak was at 56 weeks with a frequency of 32%. The first peak in survival times (24 weeks) in group 4 did not appear to differ greatly from that in group 1 (16 weeks). Even though there were no remarkable differences between these two survival times, there were differences in the frequency of patient survival in that distribution. The frequency in group 4 was about one half of that in group 1,

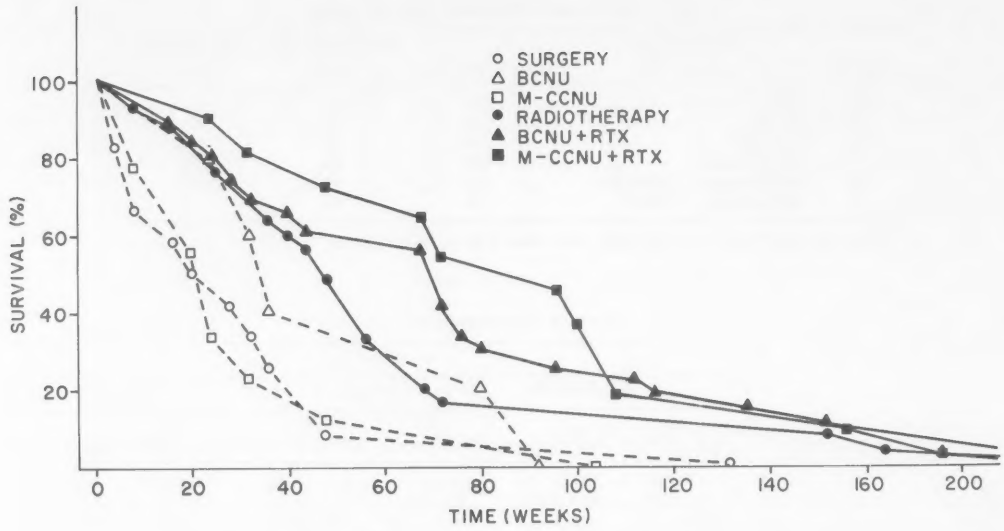

FIGURE 1.—Survival curves by Culter's life table method. M-CCNU = MeCCNU, RTX = radiotherapy.

and the survival distribution in the remaining one half (32%) shifted to the right to form a second peak of survival in the range of 56 ± 8 weeks.

The addition of MeCCNU to radiation therapy approximately doubled the duration of modal survival from 56 weeks (radiation only) to 100 weeks (radiation plus MeCCNU), with similar frequencies of 32% and 30%, respectively. Furthermore, the first peak of treatment failures in group 5 was eliminated. Although the number of patients in this group was not large, there was a change in the shape of the survival distribution curve, which may represent an improvement in the survival time following treatment of malignant gliomas (fig 3).

DISCUSSION

This study indicated the importance of radiotherapy and chemotherapy in the treatment of malignant gliomas (tables 2 and 3). In our studies, the well-established important role of postoperative radiation treatment (6) was confirmed. Interestingly, we found that when MeCCNU was combined with radiotherapy, survival distribution among patients with malignant gliomas was significantly skewed. Median survival time was 92 weeks versus 47 weeks (MeCCNU and radiation versus radiation therapy only) and 69 weeks for patients treated with radiation therapy plus BCNU. Some selection of either

TABLE 4.—Modal survival in each group

Group	Postsurgical treatment	No. of patients	Survival in wks			
			Median	Mean	Modal (% of patients) 1st	2nd
1	None	12	19	27	16 (50)	—
2	BCNU	5	30	45	32 (60)	—
3	MeCCNU	9	16	33	16 (66.7)	—
4	Radiotherapy	25	47	58	24 (28)	56 (32)
5	Radiotherapy + MeCCNU	10	92	78	—	100 (30)
6	Radiotherapy + BCNU	26	69	67.7	24 (30.7)	74 (30.7)

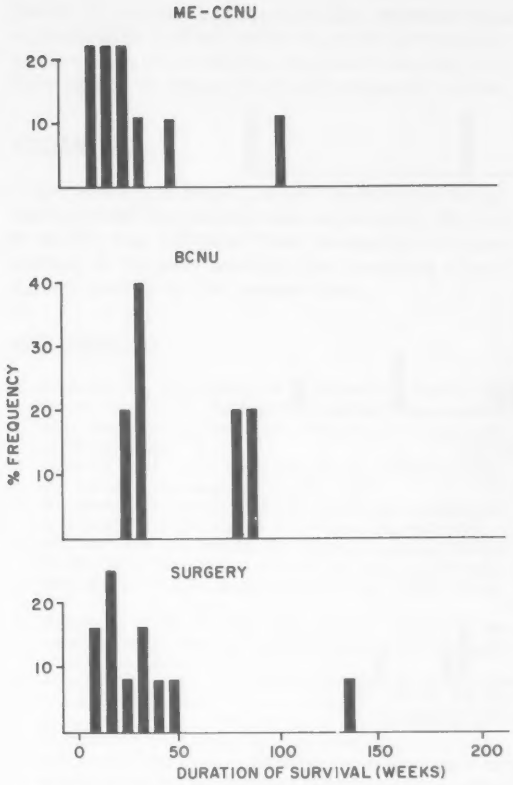

FIGURE 2.—Survival distributions in category A (without radiation treatment).

younger patients or those with lower grade tumors may have contributed to these results. Our experiences with MeCCNU plus radiation therapy suggest a difference for this agent in combined modality therapy. However, a need for more critical analysis of patient age in tumor response is necessary.

Because the University of Kentucky represents a single institution in the BTSG, certain categories received added weights in the protocol study. Randomization assignments to various categories received different weights but represented concurrently treated patients receiving identical treatment, eg, surgery, radiotherapy, and chemotherapy, as the combined modality therapies. Studies by Walker and Strike (4) have already shown that surgery was less effective alone than when combined with radiotherapy. Likewise, Walker and Gehan (7) have shown that BCNU alone is minimally effective compared to BCNU plus radiotherapy. Therefore, in these studies the groups given both chemotherapy and radiotherapy received added weight, and the surgery only or chemotherapy only groups, less weight.

The median survival times in each group (except MeCCNU plus radiotherapy) observed in this study agree with the results of others. The effect of radiation and/or MeCCNU treatment on median survival has been reported (4), but use of median survival as a parameter to demonstrate differences in response failed to show an increase in survival when MeCCNU was added to postoperative radiation treatment. Clearly, additional studies are indicated based upon the observations reported here. Bateman et al (8) have shown in cell survival studies on a human tumor xenograft grown in mice that MeCCNU reduced by a factor of 2.83 the radiation dose necessary for a fixed surviving fraction (ie, MeCCNU plus 460 rad is equivalent to 1300 rad). Thus, interaction in effect between MeCCNU and radiation is plausible.

Generally, surgery may potentially eliminate almost 90% of a removable malignant glioma, reducing the tumor mass from, for example, 100 to 10 g. The residual tumor will still contain a high log number of cells, which radiotherapy will further reduce. Wilson (9) estimated that residual mass after surgery contained 10^{10} cells. As chemotherapeutic agents generally kill the malignant cells by first-order kinetics, it is not surprising in view of this large cell number that chemotherapy alone failed to produce a significant increase in survival (category A in table 2). The role of radiation therapy is well-accepted in the management of malignant gliomas. But even with a 4–5-log kill of tumor cells, the tumor load may still greatly exceed curative numbers. An analysis by Walker et al (6) indicates a direct relationship between total dose of radiation and increased survival time for patients with malignant gliomas. However, insufficient irradiation adds little to survival of patients. Only when 6000-rad doses are employed does median survival increase compared to values in patients who have had no radiotherapy. Variations in median survival among irradiated groups in various studies are most probably due to differences in the radiation dose used. Combined management with postoperative radiotherapy and chemotherapy would be the most ideal and reasonable approach. Postoperative radiation would decrease the burden of malignant cells, and effective chemotherapeutic agents would then further decrease the load to potentially curable levels.

Nitrosoureas have been shown to have definite activity against malignant gliomas (10,11,13,14, 19–26). BCNU has been studied most extensively

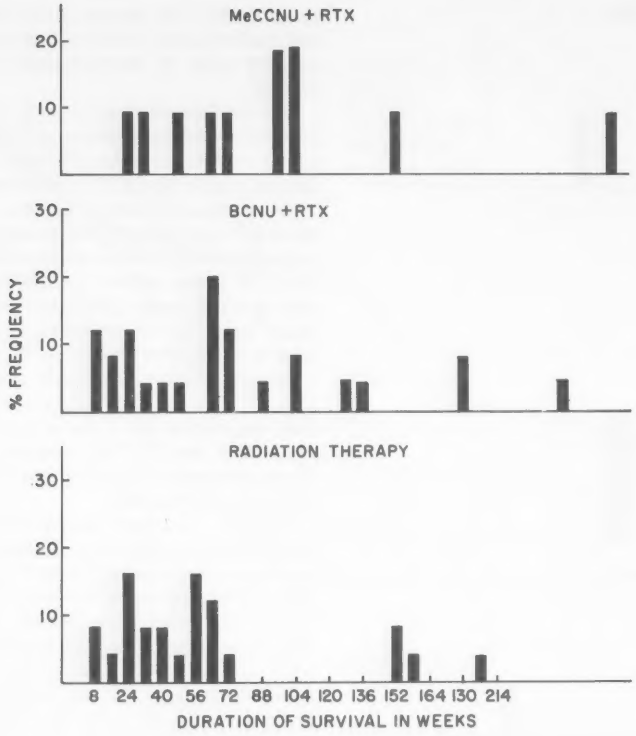

FIGURE 3.—Survival distributions in category B (with radiation treatment). RTX = radiotherapy.

and has been reported to yield a 46%–51% response rate in patients with malignant gliomas (10–18). CCNU and MeCCNU have also produced response rates similar to those of BCNU. In this study, patients receiving radiation therapy and MeCCNU had a significantly increased survival, ie, 70% 1-year survival and 30% 2-year survival (table 3), compared to all other groups. In patients receiving radiation therapy and BCNU, survival increase was only modest, ie, 61.5% 1-year survival and 26.9% 2-year survival compared to 48.0% and 16.0%, respectively, for the irradiation only group.

The feature that distinguishes these two agents was seen during the initial 6 months after surgery, when the highest mortality usually occurred. There appeared to be a biphasic distribution of survival times in the group that received BCNU and radiation therapy in contrast to the protracted survival distribution among patients receiving MeCCNU and radiation therapy (figs 2 and 3). Selection based upon patient age or tumor grade but not on surgical procedure may have accounted for the observed results. Radiation therapy was always administered to a dose of 6000 rad in 6 weeks. Radiation technique at this institution has emphasized a uniform dose and has used a calvarial-scalp compensator developed here (5). Number of chemotherapy courses may also have contributed although those who survive longer will inevitably receive a larger number of courses.

This study indicated that MeCCNU in conjunction with irradiation affected survival favorably and decreased mortality in the first 6 months following surgery. The duration of modal survival was increased nearly two times compared to irradiation only and was six times that of the surgery alone group. We propose that there is a need for further evaluation of MeCCNU and for development of additional more effective agents since the proven efficacy of the agents we used was limited to a short

period. It may also be important that radiation dose in the skull be uniform rather than 15%–20% higher in the vertex of the skull as was shown (5); this may have helped us obtain the results reported herein.

ADDENDUM

The efficacy of BCNU in the treatment of malignant gliomas has recently been reported by Walker et al (27) who indicated that postoperative radiotherapy is the best modality for treatment of malignant gliomas at the present time.

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Indicine-*N*-Oxide: A New Antitumor Agent[1]

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SUMMARY

Indicine-*N*-oxide, a pyrrolizidine derivative, was selected for development because of activity in the murine P388 leukemia model. Route and schedule dependency have been demonstrated. It is believed that the antitumor activity of the drug is mediated via antimitotic effects and chromosomal damage. However, the active metabolic species responsible for these antitumor properties is not yet known. The major toxic effect was myelosuppression. Phase I clinical trials have arrived at recommended doses for further study. Colon carcinoma has been found to be possibly responsive, and several tumor types were reported stable during phase I testing. In a single phase II study in refractory leukemia, there were three responses, including one complete response, among seven patients. Phase II studies in all panel tumors are indicated, especially colon carcinoma and leukemias.

[Cancer Treat Rep 65:53–56, 1981]

Indicine-*N*-oxide (fig 1), a pyrrolizidine alkaloid, was first isolated from *Heliotropium indicum* by Mattocks et al (1). This flower has been used medicinally in India and is an herbal treatment for warts. In addition, ingestion by grazing cattle induces both functional and pathologic changes in the liver (2). The agent is water-soluble and was selected for study because of activity against intraperitoneally implanted murine P388 leukemia. It has since undergone further preclinical evaluation and has entered phase I clinical trials. Mechanisms of the biologic antitumor activity of the drug may lie in its antimitotic and chromosome-damaging properties (2). It has been demonstrated that the mitosis usually occurring in rat hepatocytes following partial hepatectomy can be inhibited by pretreatment with pyrrolizidine alkaloids. In addition, chromosomal breakage has been demonstrated in *Drosophila* and human chromosomes (2).

Experimental Antitumor Activity

Route and schedule dependency have been shown in the P388 system.[3] The most active regimen was daily ip injection for 9 days, resulting in a 72% increase in lifespan (ILS). Sc and oral administration for 9 days were not effective. When indicine-*N*-oxide was given ip on Days 1 and 9, a 45% ILS was noted; when given ip on Day 1 only, a 27% ILS was obtained. In the Walker 256 rat carcinosarcoma model, tumor weight inhibition was demonstrated when the drug was given ip and im, but no effects were noted after oral or iv administration. In the B16 melanocarcinoma model, ILS was greatest when the drug was given ip on Days 1, 5, and 9. Again, the sc route was ineffective. Marginal activity has been seen against the L1210 and Lewis lung tumor models. Colon 26 and 38 models have proven refractory (*see* footnote 3).

Pharmacology

Ames and Powis (3) have described an electron-capture gas-liquid chromatography technique for

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[3]Clinical brochure: indicine-*N*-oxide. Prepared by the Investigational Drug Branch, National Cancer Institute, Bethesda, MD, Sept 1977.

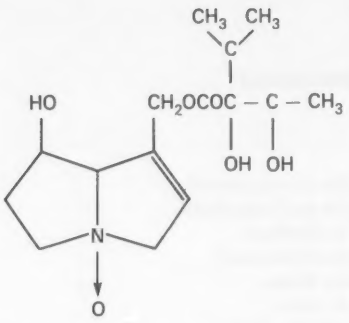

FIGURE 1.—Structure of indicine-*N*-oxide.

assaying indicine-*N*-oxide and its derivative indicine in animal and human plasma and urine. When administered iv to rabbits, a rapid biphasic disappearance pattern was noted, with a mean α-half-life of 12.8 minutes and a mean β-half-life of 70.3 minutes. Most of the intact drug was excreted in the urine within the first 2 hours following administration. Indicine continued to be excreted over the entire 24-hour urinary collection period. A slower and more variable drug disappearance pattern was noted in man (4). After iv administration of 3 g/m², the mean α-half-life was 40.3 minutes and the mean β-half-life was 85.6 minutes. Within 24 hours, 40% of the total drug was recovered as the parent compound and 2% was recovered as indicine in the urine. Kovach et al (5) found no accumulation of drug in patients treated over a 5-day period.

The metabolic pathway for indicine-*N*-oxide has been reviewed by Powis et al (6). The parent drug, when injected iv, passively diffuses into the gut where the flora reduce the drug to indicine. Indicine reaches the liver and is conjugated or oxidized to dehydroindicine and retronecine by the microsomal system. Further oxidation by the microsomal system yields dehydroretronecine, which is capable of alkylation, although this might not be the active derivative.

In studies aimed at defining the agent responsible for the antitumor activity of indicine-*N*-oxide, Powis et al (7) concluded that metabolites were less active than the parent compound itself in P388 leukemia. Other speculative proposals for the specific mechanism of action have been made. Culvenor (8) proposed that the pyrrolizidine structure, bearing similarities to purines, interferes with purine metabolism. Others have suggested that the action of *N*-oxides may be mediated through their action as oxygenating agents. Thus, the specific mechanism of activity and ultimate agent responsible for the activity still remain unknown.

Preclinical Toxicology

Dogs and monkeys were treated iv with various doses and schedules (*see* footnote 3). In dogs given a single dose, the toxic dose low (TDL) was 3000 mg/m², the toxic dose high (TDH) was 12,000 mg/m², and the lethal dose (LD) was 24,000 mg/m². When dogs were treated on a 5-day schedule, the TDL was 750 mg/m²/day, the TDH was 1500 mg/m²/day, and the LD was 3000 mg/m²/day. In rhesus monkeys treated on a 5-day schedule, the TDL was 450 mg/m²/day, the TDH was 1800 mg/m²/day, and the LD was 3600 mg/m²/day.

Toxicity was noted in several organ systems. Hematologic depression was common, with decreases in all circulating elements. Bone marrow examination revealed hypocellularity with depressed wbc and rbc precursors. Gastrointestinal toxic effects included anorexia, emesis, hemorrhagic diarrhea, and weight loss, with pathologic examination revealing mucosal degeneration. Abnormalities in renal function included increased BUN and creatinine, with proteinuria and wbcs and rbcs noted in the urine. Hepatic abnormalities were manifested by SGOT, SGPT, and sulfobromophthalein elevations; however, pathologic changes were generally absent on the sections that were reviewed.

Phase I Studies

Taylor et al (9) studied two iv schedules (weekly × 4 and intermittently every 3 weeks). On the weekly × 4 schedule, doses ranged from 1.0 to 7.5 g/m²/week. Doses from 5 to 10 g/m² were given on the every-3-week schedule. Hematologic toxic effects were dose-related with depressions of both wbcs and platelets. A maximum tolerated dose of 6 g/m²/week was recommended for the weekly schedule and 7.5 g/m² for the every-3-week program. These doses were found to produce median wbc count nadirs of 3.0 × 10^3/mm³ and 2.9–4.0 × 10^3/mm³, respectively; the corresponding median platelet count nadirs were 110–150 × 10^3/mm³ and 81–150 × 10^3/mm³. Recommended doses for phase II studies were 6 g/m² on the weekly schedule and 7.5 g/m² on the intermittent schedule for patients without heavy pretreatment. Since patients with prior nitrosourea therapy had more severe myelosuppression, their recommended doses were 4.0 g/m² weekly and 5 g/m² intermittently. The only nonhematologic toxic effects were nausea and vomiting.

Kovach et al (4,5) administered total doses of 0.75–15 g/m² divided over 5 days. The dose-limiting toxic effect was myelosuppression, which was dose-dependent. The median blood cell count nadirs were

on Day 20 for leukopenia and on Day 18 for thrombocytopenia. Patients with prior nitrosourea therapy had more severe myelosuppression, and a trend toward cumulative myelotoxicity was also observed. Nonmyelosuppressive toxic effects included infrequent and transient elevations of liver functions, reversible increases in serum creatinine, and possible hemolysis. The recommended starting dose was 3 g/m²/day for 5 days in patients without prior nitrosourea therapy, with courses repeated every 5–6 weeks. Although a recommendation for patients previously treated with nitrosoureas was given (2.25 g/m²/day for 5 days every 5–6 weeks), subsequent investigation has shown that even this lower dose may lead to excess myelosuppression in this group of patients. Therefore, a final dose and schedule for such patients has not yet been derived but is probably < 2.25 g/m²/day for 5 days.

Ohnuma et al (10) administered the drug iv over 15–30 minutes every 3 weeks at doses ranging from 1 to 7 g/m². Hematologic toxic effects, including anemia, were dose-related and dose-limiting, with evidence of cumulative bone marrow effects in patients receiving multiple courses. Nonhematologic toxic effects included nausea, vomiting, and hepatic dysfunction. Interestingly, hepatic dysfunction was linked to more pronounced hematologic toxicity whether the hepatic dysfunction was present before or after treatment with indicine-*N*-oxide.

Among 23 patients with colon carcinoma, Taylor et al (9) reported two with partial responses and 11 with stable disease. Stable disease was also reported in a small number of patients with salivary gland, breast, non-small cell lung, and ovarian carcinomas. One patient with leiomyosarcoma was also reported with stable disease. Kovach et al (4,5) found no tumors to be responsive. However, short periods of stable disease were reported for patients with colon, esophagus, and pancreatic carcinomas. One patient with colon cancer was reported to have stable disease for > 6 months. In Ohnuma's study, no complete or partial responses were noted; however, improvement was found in one patient with a skin melanoma (10).

Phase II Studies

Only one phase II study of indicine-*N*-oxide has been reported to date: that by Letendre et al (11) in patients with refractory leukemia. Seven patients (five with acute lymphocytic leukemia, one with acute myelocytic leukemia, and one with acute myelomonocytic leukemia) were given a dose of 3.0 g/m²/day × 5 days. Responses were noted in three of the patients with acute lymphocytic leukemia. A 4-year-old child in multiple relapse achieved a complete response lasting 8 weeks; a 15-year-old child in his second relapse had a partial response; and an 11-year-old child, also in his second relapse, experienced peripheral and marrow hypoplasia but did not benefit clinically and died. Other than myelosuppression, mild nausea was the only toxic effect observed.

DISCUSSION

Indicine-*N*-oxide is a drug with several features of clinical interest. It is highly active in the murine P388 leukemia and also may have mechanisms of action (antimitotic and chromosome-damaging properties) that would avoid cross-resistance with those of many established chemotherapy agents. Thus, indicine-*N*-oxide may prove to be a useful drug for combination chemotherapy studies, if activity in phase II trials can be demonstrated.

The clinical evaluation of indicine-*N*-oxide is clearly at an early stage. The drug has shown hints of activity in the usually resistant solid tumors, ie, colon carcinoma and malignant melanoma. This antitumor activity occurred in patients who were all heavily pretreated before receiving indicine-*N*-oxide therapy. Therefore, it would be appropriate to evaluate the drug in untreated patients with these advanced solid tumors. The activity of the drug in patients with advanced leukemia is also of considerable interest. If further evaluation confirms that indicine-*N*-oxide is capable of inducing complete remissions in heavily pretreated leukemia patients, the drug should be considered for potential use in combination chemotherapy regimens early in the course of acute leukemia. Caution is advised in treating heavily pretreated patients or those who received prior nitrosourea therapy, since these patients may develop severe myelosuppression.

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"High-Dose" Thymidine in Clinical Oncology[1]

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Thymidine (TdR) (fig 1) is an endogenous nucleoside that serves as a DNA precursor, being incorporated in the forming DNA structure as thymidine triphosphate (dTTP). dTTP originates from uridine diphosphate (UDP) or cytidine diphosphate (CDP) through the common formation of deoxyuridine monophosphate (dUMP), which is converted to thymidine monophosphate (dTMP) by the action of thymidylate synthetase and by the phosphorylation of TdR itself to the diphosphate and triphosphate forms by the catalytic reaction of kinase enzymes (1) (fig 2).

Exogenous addition of high-dose TdR results in an increase in the dTTP pool that promotes an expansion of deoxyguanosine triphosphate (dGTP) and a reduction of the deoxycytidine triphosphate (dCTP) pool. Variation in the size of the latter closely reflects the rate of DNA synthesis. The biochemical events underlying this nucleotide pool imbalance seem related to the dTTP negative feedback regulation of several enzymes involved in the synthesis of nucleotides, including allosteric regulation of the ribonucleotide reductase enzyme system that catalyzes reduction of ribonucleotides to deoxyribonucleotides. Binding of dTTP to ribonucleotide reductase forms a dTTP-enzyme complex that promotes reduction of guanosine diphosphate (GDP) to deoxyguanosine diphosphate (dGDP). This increases the dGTP pool but inhibits CDP reduction to deoxycytidine diphosphate (dCDP), thus decreasing the dCTP pool and precluding DNA synthesis and consequent cell division (2–19).

Addition of deoxycytidine reverses the TdR inhibitory effects on DNA formation and permits resumption of cell proliferation (12,20,21). The plasma level of deoxycytidine in the rat is 0.02–0.04 mM (22), a level sufficient to reverse the effect of 1 mM TdR in Novikoff hepatoma cell cultures. In healthy persons, and in burned or cancer patients, deoxycytidine levels are negligible (< 0.03 μg/ml or 1.3 × 10^{-4} mM) and therefore unable to reverse the effect of exogenous TdR (23).

PRECLINICAL STUDIES

Since the initial description by Xeros (24), excess TdR has been used in concentrations of ≈ 10^{-2}–10^{-5} M as an in vitro cell synchronizing agent (10–12,25–32). The addition of TdR to cell culture systems produces mitotic arrest in the G_1 and early S phase of the cell cycle. There is a resumption of mitosis in a partially synchronized burst after the removal of the TdR. Of particular interest in cancer chemotherapy is the cytotoxicity that occurs when the TdR exposure is longer than one generation time or when the exposed cell line is derived from a TdR-sensitive tumor (18,21,30,33–35).

An explanation of the differential TdR sensitivity of the various cell lines derived from the same or different malignant tumor types may prove critical in the rational development of TdR therapy. Reynolds et al (36) have found evidence indicating that the level of dCTP correlates with the TdR sensitivity of cells. Less sensitive cells contain more dCTP than do the highly sensitive cells. The difference in dCTP pools could be related to the differential activity of any of the enzyme systems involved in the dTTP synthetic pathway. Of interest is the recent report by Kufe et al (37) concerning the correlation of clinical therapeutic effect in acute myelocytic leu-

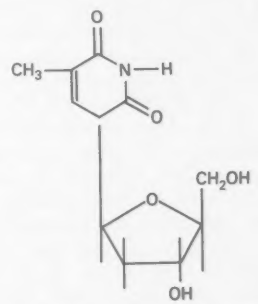

FIGURE 1.—Structure of TdR.

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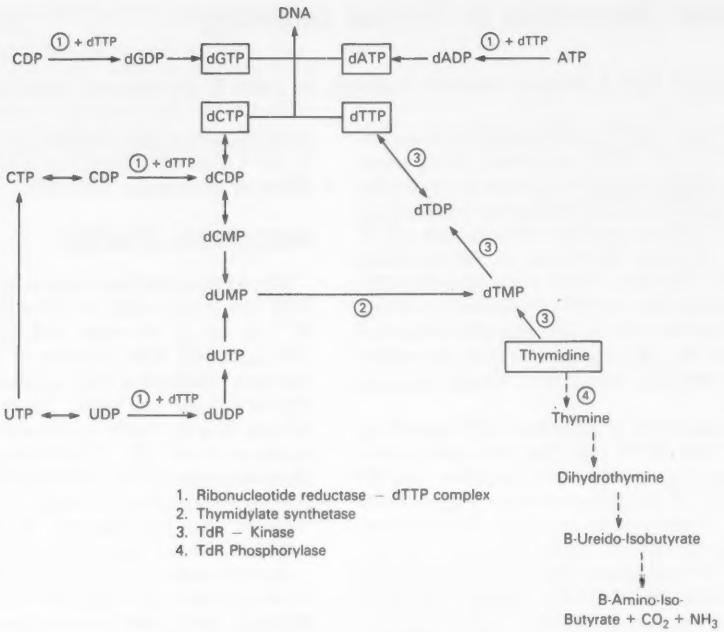

FIGURE 2.—DNA metabolic regulation.

kemia (AML) and T-cell acute lymphocytic leukemia (ALL) with TdR kinase-phosphorylase enzyme levels. They showed that these cells had up to tenfold higher levels of TdR kinase and up to 30 times lower levels of TdR phosphorylase than did malignant B lymphocytes from lymphoma patients in leukemic phase. In addition, the relative enzyme ratios were predictive of the dTTP formation from TdR.

The issue of TdR sensitivity is compounded by the development of TdR-resistant mutants. Zielke (38), studying lymphoid cell lines derived from a patient with ALL, estimated the frequency of mutation to be ≈ 1 in 100,000 cells (39,40). Of additional importance is the selective differential sensitivity between malignant and benign cell lines of the same histologic origin, first reported by Lee et al (34), which demonstrated a selective killing of malignant human (colon carcinoma and melanoma) and mouse (neoplastic mesenchymal) cell lines when exposed to excess TdR at 1–10 mg/ml. In contrast, nonmalignant human (melanocytic and intestinal epithelium) and mouse (benign mesenchymal) cell lines were not as sensitive to these TdR concentrations. Less than 23% of the tumor cells survived 72 hours whereas ≥ 60% of the nonmalignant cells survived. In addition, clonogenicity assays showed differences in cloning efficiency between normal and malignant cells after exposure to TdR. There was 95% inhibition of cloning efficiency in malignant cells versus 66% inhibition in normal cells.

The differential effect of TdR in the survival of normal and cancerous cells has been confirmed by Tsuboi and Kwong (41), utilizing normal human diploid and colonic adenocarcinoma cell lines. Normal cells seem to enter a quiescent state due to growth arrest, whereas cancer cells continue dividing at the expense of a limited survival and death. Similarly, Tobey et al (42) demonstrated differential TdR response between the parent BHK21 hamster cell line and its polyoma-transformed derivative.

Lee et al (43) expanded their experience to an in vivo human xenograph in nude (athymic) mice. The continuous sc infusion of TdR at 1 g/kg/day over 72 hours for three cycles, separated by a 4- or 5-day rest period between doses, produced growth inhibition of a human melanoma tumor. An additional significant increase (25%) in survival time for the TdR-treated mice over that of the control was recorded. The same investigators extended their studies to other human tumor heterotransplants in

nude mice, utilizing TdR at 12–24 g/kg/day as a continuous infusion for 3–6 days. Courses were separated by a 3–6-day rest period, and a minimum of two and a maximum of eight cycles were administered. Complete regression was achieved in seven of nine human tumor xenografts, including five melanomas originated from different cell lines, one teratocarcinoma, and one oat cell carcinoma. Two partial regressions in breast cancer were also noted (44,45).

Howell et al (46), who studied human tumor xenografts in nude mice utilizing a similar TdR dose of 1 g/kg/hour as a continuous 72-hour infusion repeated every 3 weeks, were unable to confirm the results of Lee et al (43). A significant growth inhibition (cytostatic rather than cytocidal) was seen only in melanoma, and slowing of the growth of an oat cell tumor was recorded. No antitumor effect was noted for breast and colon tumors. In addition, TdR was ineffective in other transplantable tumor systems, ie, L1210 leukemia, Lewis lung carcinoma, and colon tumors 26 and 38. However, the latter studies did not employ repeated courses of infusion and used lower doses than in the xenograft tests (J. Venditti, personal communication). Semon and Grindey (47) also reported no effect on L1210 leukemia utilizing a continuous infusion of TdR at 18 g/kg/day for 96 hours.

Reiter (48) studied the effect of intermittent ip injection of TdR in mice inoculated ip with TdR-sensitive EL-4 thymoma cells. This investigator noted a significant prolongation of animal survival and obtained "cures" in three of ten mice receiving a small inoculum of malignant cells.

Animal toxicity studies with high-dose TdR have been performed (49). The acute lethal toxicity of single ip injections of TdR in CDF male mice was studied; the LD50 was 4.075 g/kg (12 g/m²). The animals became comatose and died within 24 hours after TdR administration. The TdR plasma concentration was lethally toxic at 10 mM when this level was maintained for 20 hours. In addition, furosemide at 8 mg/kg was shown to increase toxicity and NaCl decreased it.

CLINICAL STUDIES

TdR Pharmacokinetics in Man

TdR is normally present in human serum (50,51) in a range of 0.1–0.76 μM, with a median of 0.19 μM as determined by radioimmunoassay (50). TdR is cytocidal in vitro and in animals when given in sufficient quantity to produce a plasma TdR concentration near or at the millimolar level. In man, this level can be achieved without serious deleterious effects (37,52–55).

Exogenous TdR pharmacokinetics vary with the administered dose. At a dose of 8 g/m²/day, the plasma TdR concentration increases sixfold to eightfold to 1–1.5 μM with a TdR half-life of 8–10 minutes, regardless of whether a pulse dose or a continuous infusion over 3 days is given. TdR concentration in the cerebrospinal fluid reaches an equilibrium with that of the plasma in 2 hours. Less than 2% of the administered TdR is recovered in the urine, suggesting that 98% is metabolized further, most likely in the liver (53,56). At a higher dose, in the range of 64–128 g/m²/day, the TdR plasma concentration increases to the millimolar level with a plasma half-life that varies both as a function of the infusion time (TdR half-life of 10, 80, and ≈ 100 minutes for infusions lasting 30 minutes, 8 hours, and 24 hours, respectively) and also as a function of the dose (TdR half-life of 7.5 and 106 minutes with TdR doses of 2 and 30 g/m², respectively) given as a rapid infusion (55–57). The renal clearance of TdR ranged from 41% to 67% of total body clearance, suggesting a saturation of the metabolic handling of TdR followed by the elimination of the unmetabolized portion through the kidneys (58). Thymine half-life is significantly longer than that of TdR, and concentration of thymine (unlike TdR) may remain constant after the end of the infusion, pointing again to a saturable metabolic mechanism. Renal clearance for thymine accounts for 13%–18% of the total body clearance (59).

Ensminger and Frei (60) studied the hepatic extraction of TdR using the iv route and direct hepatic artery infusion. They found that the hepatic TdR extraction expressed as a ratio of hepatic artery level:hepatic vein level was high, ranging from 0.64 to 0.85 with doses up to 16 g/m²/day and becoming progressively less with doses of 16–128 g/m²/day when the iv route of administration was used. Likewise, with the hepatic artery infusion route, the TdR extraction was elevated (94%–98%) at 8 g/m²/day and progressively decreased as doses were increased to 16 and 32 g/m²/day. The authors concluded that the hepatic artery extraction of TdR, although efficient, is saturable, and the level of TdR in the millimolar range (cytotoxic level) can be achieved in the liver while nontoxic levels are present in the systemic circulation when the hepatic artery route is used. This would be of potential use in the treatment of liver metastasis.

Clinical Trials

TdR has been used in human subjects for a number of years in the tritiated form for pharmacoki-

netic and cytokinetic studies with no toxicity noted (61). Similarly, nonradioactive TdR has been intensively used in the treatment of megaloblastic anemias with total doses of up to 9 g given iv with remarkably low toxicity. Only occasional phlebitis and a "slight collapse" in a patient after im injection of the first 200 mg of TdR were reported (62–68). Besides the possible toxicity of TdR, the large volume of fluids necessary for dissolving the relatively insoluble TdR is a problem. The maximum amount of TdR that may be dissolved in 1 liter of solution is 30 g.

The Division of Cancer Treatment of the National Cancer Institute (NCI) has sponsored numerous clinical trials of TdR (table 1), and preliminary findings for some of these trials have been published. Woodcock et al (55) of the Memorial Sloan-Kettering Cancer Center, New York, NY, reported their experience using TdR in doses ranging from 40 to 90 g/m²/day as iv continuous infusion for 5 days, with a 5-day rest period. A total of eight patients (two with AML, two with ALL, two with lung cancer, one with breast cancer, and one with renal cell cancer) received 14 courses. All patients with a normal bone marrow manifested blood cell count depression; other toxic reactions were anorexia, lethargy, and diarrhea. In one patient with a TdR plasma level of 3 mM, vomiting, partial alopecia, and somnolence were noted. Two leukemia patients developed a marked decrease in peripheral blast cells, but no bone marrow hypoplasia was observed.

Chiuten et al (52) at the Baltimore Cancer Research Program, NCI, reported on ten patients treated with TdR at 75 g/m²/day given as a continuous iv infusion for 5 days, repeated every 10 days. The types of malignancies were two acute nonlymphocytic leukemias, one Hodgkin's disease, two melanomas, three colon cancers, one metastatic adenocarcinoma of unknown origin, and one oat cell cancer. Toxic effects included bone marrow depression with an average nadir for leukocytes, platelets, and hematocrit of $2.5 \times 10^3/mm^3$, $97.5 \times 10^3/mm^3$, and 27.3%, respectively. Other toxic effects were nausea, vomiting, anorexia, diarrhea, indigestion, somnolence, headaches, visual illusions, and memory impairment. TdR crystals were noted in the urine of all patients after refrigeration. Tumor regression (less than partial response) occurred in one melanoma patient, with decrease in the size of subcutaneous nodules. Another patient with acute nonlymphocytic leukemia had clearing of peripheral blast cells and decreased hepatosplenomegaly, but without bone marrow changes.

Kufe et al (37) at the Sidney Farber Cancer Institute carried out a clinical trial utilizing TdR in a similar schedule and dose. Eleven patients, including three with ALL (T-cell), four with AML, two with poorly differentiated lymphomas in leukemic phase, one with diffuse histiocytic lymphoma, and one with lymphoblastic lymphoma, were treated with a total of 15 courses. Associated toxic reactions were headache, anorexia, nausea, vomiting, and diarrhea. All of these toxic effects occurred within 24 hours after the TdR infusion began. Crystals precipitated from the urine stored at 4°C and were even noted at room temperature. Two patients had no bone marrow involvement, but both developed leukopenia and one also had thrombocytopenia. Sequential bone marrow aspiration showed no changes. Antitumor response was suggested in five of the 11 patients. The three patients with T-cell ALL showed a rapid decrease in peripheral blast cells. In one patient, this was associated with a decrease in hepatosplenomegaly and lymphadenopathy, and the marked improvement of a diffuse leukemia cutis. No changes in the bone marrow were noted. Another of the T-cell ALL patients showed a decrease from 90% to 40% in the number of bone marrow blast cells after 7 days of treatment, but again no bone marrow remission was observed. Among the four AML patients, two had abrupt decreases in the peripheral blast cell count, but none achieved a bone marrow remission. The two patients with B-cell, poorly differentiated, lymphocytic lymphoma in leukemic phase and the one with diffuse histiocytic lymphoma with bone marrow involvement only

TABLE 1.—NCI-sponsored clinical studies with high-dose TdR

Institution (ref No.)	TdR treatment plan
Baltimore Cancer Research Program, NCI (52)	75 g/m²/day continuous iv infusion × 5 days, every 10 days
Sidney Farber Cancer Institute (37)	75 g/m²/day continuous iv infusion × 5 days, every 10 days
Memorial Sloan-Kettering Cancer Center (55)	40–90 g/m²/day continuous iv infusion × 5 days, every 10 days
Stehlin Foundation	45–90 g/m²/day continuous iv infusion × 5 days, every 10 days
University of Michigan	32–128 g/m²/24 hrs iv infusion 32–128 g/m²/24 hrs iv infusion, followed by a 2nd dose after 12–24 hrs rest 32–128 g/m²/day continuous iv infusion with progressive 24-hr increments 16–32 g/m²/day continuous hepatic artery infusion for 5 days

showed a very gradual decline in peripheral blast and normal cells, unlike the ALL and AML patients, suggesting a different response according to cell types.

FUTURE PROSPECTS

The clinical trials, to date, have confirmed the prediction that a millimolar concentration of TdR can be achieved with tolerable toxicity. No data are available on long-term toxicity. This is a point of concern since chronic side effects are an important consideration if leukemia and lymphoma patients with the possibility of long survival are to be treated. It has been demonstrated that chromosomal abnormalities may appear with in vitro exposure of cells to excess TdR (30,69,70). The evidence that TdR may be cytocidal to neoplastic cells has not been encouraging. The one cell type that appears to respond is the leukemic blast cell, with the T-cell type possibly being the most sensitive. More work needs to be done in this area. In view of the massive quantities of TdR required and the associated large volumes of administrations, the evaluation of TdR will move at a relatively slow pace. Unless some very dramatic clinical efficacy is demonstrated, its potential as a single therapeutic agent appears not to be great.

Some alternate strategies for clinical evaluation of TdR are possible. Limiting patient selection to those whose tumors are shown to be sensitive in cell culture may hasten and improve its evaluation. Other possibilities might include preparing more soluble forms of TdR or using inhibitors of TdR phosphorylase to prevent the metabolism of TdR to thymine. It may be that the future clinical role of TdR will be in combination chemotherapy regimens. TdR would be used for its potential to modulate the biochemical activity of antimetabolites such as the fluorinated pyrimidines (71–78), cytarabine (78,80), and methotrexate (47,53,56,81,82).

Much excitement was generated over the potential of high-dose TdR, but the clinical trials have failed to fulfill the initial hopes for this drug. However, it should be emphasized that these trials are still at an early stage. The whole saga of high-dose TdR therapy should be an object lesson to the lay and scientific communities. It emphasizes the need for careful evaluation of all purported "breakthroughs" in cancer therapy through carefully designed clinical trials before widely disseminating information on such treatment.

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Trials of Single-Agent and Combination Chemotherapy for Advanced Cancer of the Pancreas[1,2]

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SUMMARY

In a study of 127 patients with advanced measurable pancreatic cancer, objective response rates of 2% (melphalan), 10% (5-FU and methyl-CCNU), and 7% (5-FU, methyl-CCNU, and streptozocin) were achieved. No responses occurred in the 28 patients treated with VP-16-213 who had already received prior chemotherapy. Toxicity was greater for the two- and three-drug combinations than for melphalan. There was no difference in survival following use of the three treatment regimens. Further studies are needed to develop effective chemotherapy for patients with cancer of the pancreas.

[Cancer Treat Rep 65:65–68, 1981]

Conventional chemotherapy for advanced carcinoma of the pancreas produces a low incidence of objective response (1). The purpose of this study was to determine the toxicity and degree of antitumor activity in previously untested agents and combinations that included melphalan, 5-FU, and methyl-CCNU (MeCCNU), and 5-FU, MeCCNU, and streptozocin in patients with advanced measurable pancreatic adenocarcinoma. A crossover design for patients with prior 5-FU and nitrosourea therapy, or for following disease progression on initial therapy, allowed evaluation of the synthetic podophyllin compound, VP-16-213.[3]

MATERIALS AND METHODS

One hundred and fifty-three patients with histologically documented residual, recurrent, or metastatic pancreatic carcinoma with measurable parameters of disease who gave informed consent

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[2]This study was conducted by the Eastern Cooperative Oncology Group (*Paul P. Carbone*, chairman, CA-21115) and was supported by Public Health Service grants (CA) from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services. The following members participated in the study: Albany Medical College, NY (*J. Horton*, CA-06594); Sidney Farber Cancer Institute, Boston, MA (*R. D. Gelber*, CA-23318); American Oncologic Hospital, Philadelphia, PA (*P. Engstrom*, CA-18281); University of Pretoria, South Africa (*G. Falkson*, CA-21692); Mayo Clinic, Rochester, MN (*C. Moertel*, CA-13650); Jefferson Medical College, Philadelphia, PA (*H. Brodovsky*, CA-14215); and Roswell Park Memorial Institute, Buffalo, NY (*H. Douglass*, CA-12296). Other participating institutions include: Albert Einstein College of Medicine, Bronx, NY (CA-14958); University of Alberta, Edmonton, Canada; Baystate Medical Center, Springfield, MA (CA-20182); Brown University, Providence, RI (CA-15947); Case Western Reserve University, Cleveland, OH (CA-14548); Chicago Medical School, IL (CA-14144); Georgetown University School of Medicine, Washington, DC (CA-02824); Hahnemann Medical College, Philadelphia, PA (CA-13611); University of Florida-Jacksonville (CA-19819); Johns Hopkins Oncology Center, Baltimore, MD (CA-16116); University of Minnesota, Minneapolis (CA-20365); Mt. Sinai Medical Center, New York, NY (CA-17152); National Cancer Institute-Veterans Administration Medical Oncology Branch, Washington, DC; New York University Medical Center, NY (CA-16395); Northwestern University Medical Center, Chicago, IL (CA-17145); University of Ottawa, Ontario, Canada; Hospital of the University of Pennsylvania, Philadelphia (CA-15488); University of Rochester Cancer Center, Rochester, NY (CA-11083); Rush-Presbyterian-St. Luke's Medical Center, Chicago, IL (CA-10948); Swiss Group, Bern, Switzerland; SUNY-Stonybrook (Veterans Administration Hospital), Northport, NY (CA-20161); Tufts University, Walpole, MA (CA-07190); Natalie Warren Bryant Cancer Center, Tulsa, OK; Medical College of Virginia, Richmond (CA-10572); and Wisconsin Clinical Cancer Center, Madison (CA-21115).

[3]Handelsman H, Goldsmith MA, and Slavik M. Clinical brochure: VP-16-213. Prepared by the National Cancer Institute, Bethesda, MD, Apr 1974.

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were entered in the study. Contraindications to patient entry included major surgical procedures within 3 weeks, exploratory procedures within 2 weeks, prior chemotherapy or irradiation within the previous month, prior VP-16-213 or melphalan chemotherapy, an active infectious disease process, severe malnutrition (an intake < 1500 calories/day), leukopenia < 4500 cells/mm^3, thrombocytopenia < 150,000 cells/mm^3, or evidence of significant renal or liver function impairment. Safety of doses for three-drug combinations had been established by a pilot study performed at the Mayo Clinic (C. Moertel, personal communication).

One hundred and forty patients with no prior 5-FU or nitrosourea therapy were randomized to one of the treatments shown in table 1. Stratification variables included site of primary disease (head vs body and/or tail), grade of anaplasia, and performance status. Thirteen patients who had received prior 5-FU or nitrosourea therapy were assigned directly to VP-16-213. Fifteen patients were either cancelled or were found to be ineligible for study.

Reasons for cancellation included patient's deterioration prior to treatment (one patient), refusal of treatment (one), nonprotocol treatment (one), and insufficient data (five). In addition, three patients had nonmeasurable disease, three had initial laboratory values that did not meet study requirements, and, in one, the primary site was not the pancreas. A total of 127 randomized patients are evaluated in this report.

Ten patients who initially received melphalan subsequently received the three-drug combination. A total of 28 evaluable patients received VP-16-213: nine after 5-FU plus MeCCNU, eight after 5-FU, MeCCNU, and streptozocin, and 11 who had received 5-FU or a nitrosourea but not in this study.

PATIENT CHARACTERISTICS

Sixty-four percent of the randomized patients were male, and 59% had an initial Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1. Forty-three percent had the primary disease located in the head of the pancreas, and 57% had liver metastasis. Three patients had had prior chemotherapy, seven had had prior radiation therapy, and 12 had had a prior surgical attempt at cure. The median time from initial diagnosis to entry in the study was 5.6 weeks. The percents of patients with an excellent performance status (ECOG, 0 or 1) were 56% for melphalan, 66% for 5-FU and MeCCNU, and 56% for 5-FU, MeCCNU, and streptozocin. The percents of patients without liver involvement were 30%, 34%, and 35%, respectively.

RESPONSE

Two patients had a complete response and six others had a partial response, for an overall rate of 6%. Complete response was defined as the disappearance of all clinically detectable tumors and partial response was defined as a reduction by at least 50% of the product of the longest perpendicular diameters of the most clearly measurable tumor lesions. A 30% reduction of the sum of liver measurements below each costal margin at the midclavicular lines and xiphoid process was required if

TABLE 1.—Treatment schema and dose schedules used

	Dose schedule	
	Initial	Crossover (disease progression)
No prior 5-FU or nitrosourea therapy (randomized)		
5-FU + Streptozocin + MeCCNU	350 mg/m^2/day iv, Days 1–5 and 400 mg/m^2/day iv × 5, Days 36–40; 400 mg/m^2/day iv, Days 1–5; 150 mg/m^2 orally, Day 1	Repeat every 10 wks → Crossover to VP-16-213
5-FU + MeCCNU	350 mg/m^2/day iv, Days 1–5 and 400 mg/m^2/day iv × 5, Days 36–40; 150 mg/m^2 orally, Day 1	Repeat every 10 wks → Crossover to VP-16-213
Melphalan	6 mg/m^2/day orally, Days 1–5, repeated every 6 wks	→ Crossover to 5-FU + streptozocin + MeCCNU
Prior 5-FU or nitrosourea therapy		
VP-16-213	125 mg/m^2 iv every other day × 3 doses, repeated every 4 wks	→ Disease progression → Discontinued from the study

TABLE 2.—Objective responses of patients in the randomized initial study phase

Treatment	No. of complete + partial responses/ No. of patients treated	95% confidence intervals for response rates	Duration of response (wks)*
Melphalan	1†/43 (2%)	0–13%	43†
5-FU + MeCCNU	4†/41 (10%)	3%–23%	15,† 6, 21, 35
5-FU + MeCCNU + streptozocin	3/43 (7%)	2%–19%	9, 15, 27
Total	8/127 (6%)	3%–12%	

*CRs are given first.
†Indicates 1 complete response.

hepatomegaly was the primary indicator. Table 2 shows the incidence of responses by treatment. Most responses were in patients with abdominal or pancreatic tumors. Although liver involvement was present in four patients who responded, the liver was used as the measurable indicator site for only one patient whose liver disease completely disappeared. It may be significant that five of the eight responders were noted to have an "undifferentiated" cytopathologic tumor type. This histologic type was reported for 22% of patients entering the study.

No responses occurred in the 28 patients who received VP-16-213, either initially or following crossover.

Median survival, determined by the Kaplan-Meier method, was measured from the time of randomization. The overall median survival time was 3 months. Marginal significance tests were performed using a log-rank test. A good initial performance status and the absence of liver metastasis were the two most significant favorable prognostic indicators for survival.

Median survival times for melphalan, 5-FU and MeCCNU, and 5-FU, MeCCNU, and streptozocin were 8, 14, and 12 weeks, respectively. When these figures were corrected for initial performance status and liver involvement, using a Cox proportional hazards regression model, the median survival times were 10, 13, and 12 weeks, respectively; these are not statistically different. The median survival time from start of treatment with VP-16-213 was 9 weeks.

TOXICITY

The incidence and severity of treatment-related toxic effects of the randomized treatments are summarized in table 3. Hematologic toxicity was predominantly leukopenia, although thrombocytopenia occurred in all three treatment schedules. Toxic effects, other than hematologic or vomiting, included five cases of moderate diarrhea (one with melphalan, three with the two-drug regimen, and one with the three-drug regimen). Two cases of severe stomatitis occurred, and one patient had severe necrosis of the hand following infiltration of streptozocin. The other toxic effects were, at worst, mild or consisted of infections or bleeding associated with hematologic toxicity. Renal toxicity was not reported.

Of the 24 evaluated patients who received VP-16-213, one had life-threatening toxicity and six had severe hematologic toxicity. Three of five patients who had a bilirubin > 1 mg/100 ml had grade 3 hematologic toxicity, compared with four of 19 whose bilirubin level was less.

DISCUSSION

This study again highlights the poor prognosis of patients with pancreatic carcinoma. It is evident that melphalan is essentially ineffective in producing objective responses, and the addition of streptozocin to the combination of 5-FU and MeCCNU did not enhance the low effectiveness of 5-FU and MeCCNU alone. Although VP-16-213 did not induce a response in the 28 patients treated, it must be emphasized that all the patients had received prior chemotherapy, and the majority had a poor performance status. It is possible that the drug might be effective if used as initial chemotherapy in patients with a good performance status. An attempt was made to determine whether an elevation of the serum bilirubin was associated with a higher incidence of hematologic toxicity with VP-16-213 since this drug is presumed to be excreted by the liver. There is a suggestion that those patients with a

TABLE 3.—Treatment-related toxic effects for the randomized study

	Total No. of evaluable patients			
	Melphalan (39 patients)	5-FU + MeCCNU (37 patients)	5-FU + MeCCNU + streptozocin (41 patients)	Total (117 patients)
Worst-degree toxicity				
Drug-related death	0	1	1	2
Life-threatening	0	2	4	6
Severe	3	9	9	21
	8%	32%	34%	25%
Moderate	5	10	14	27
Mild	8	10	9	26
None	23	5	4	31
Hematologic toxicity				
Life-threatening	0	3	4	7
Severe	2	8	8	18
	5%	30%	29%	21%
Moderate	4	5	5	14
Mild	8	7	8	26
None	27	14	16	57
Vomiting				
Severe	1	3	3	7
	3%	8%	7%	6%
Moderate	2	12	15	29
Mild	4	10	15	29
None	32	12	8	52

bilirubin > 1 mg/100 ml had a higher incidence of toxicity, but the numbers are small, and the results are confounded by the fact that many patients who had the higher bilirubin also had a poorer performance status.

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Serum Concentrations of 5-FU, Ftorafur, and a Major Serum Metabolite Following Ftorafur Chemotherapy[1]

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SUMMARY

Daily 24-hour serum levels of ftorafur (FT), 5-FU, and a major FT metabolite, dehydroftorafur (DFT), were monitored by high-performance liquid chromatography as part of a phase I study designed to evaluate FT as a radiosensitizing 5-FU pro-drug in patients with advanced gastrointestinal cancers. At a daily iv bolus FT dose of 1.0 g/m², 5-FU was not detected in serum concentrations above the reliable assay limits of approximately 25 ng/ml; FT did not generate the extracellular (serum) 5-FU concentrations required for sensitization by 5-FU per se. DFT was present in every patient serum tested and was confirmed to be a metabolite of FT by in vitro conversion to 5-FU. Chemical ionization solid-probe mass spectrometry of the DFT metabolite indicates the dehydro FT structure proposed by other researchers. In six of eight patients monitored, a consistent relationship was noted between serum levels of FT and DFT.

[Cancer Treat Rep 65:69–72, 1981]

The recent development of the antineoplastic drug ftorafur (FT) has added new potential for the treatment of gastrointestinal cancers on an outpatient basis. The overall efficacy and toxicity of this drug administered in iv bolus form appear strikingly similar to those of infusional 5-FU (1). In recent trials, infusional 5-FU has augmented the effectiveness of radiation in some advanced gastrointestinal malignancies including those of the esophagus (2) and anus (3) and in colonic liver metastases (4). This finding led to clinical trials at the Veterans Administration Medical Center with bolus FT combined with radiation therapy in an effort to develop a successful cost-effective outpatient regimen. Because of the clinical similarity of FT bolus doses to continuous 5-FU infusion in patients, it has been postulated that the cytotoxic mechanism of FT involves the slow release of 5-FU into the systemic circulation (5). This theory gained support when 24-hour serum levels of 5-FU in initial bolus FT studies were reported to be similar to the 5-FU levels measured in 5-FU infusion studies (6,7). However, in a more recent report, very low circulating 5-FU levels were demonstrated and did not persist in measurable quantities (> 20 ng/ml) for > 8 hours after the 2 g/m² FT bolus dose (8,9). In view of the divergence in previously reported serum concentrations of 5-FU, serum levels of FT and 5-FU were monitored on a preliminary basis at 24 hours after the FT dose in conjunction with the ongoing trial of combined FT and radiation therapy for advanced gastrointestinal cancers. Our preliminary serum data are not sufficient to determine radiation effects on FT pharmacology but clearly indicate that 5-FU is not present in the serum of our patients in quantities comparable to those after 5-FU infusion. Additionally, an apparent dehydro metabolite of FT (DFT) appears to be present in serum in much greater quantities than are other FT metabolites so far detected (8,9); DFT was also investigated during this study.

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MATERIALS AND METHODS

FT was obtained from the Division of Cancer Treatment, National Cancer Institute, Bethesda, MD. 5-FU and thymine used as analytic standards were obtained from Sigma Chemical Co, St. Louis, MO. All solvents were of nanograde quality.

Blood samples were taken from seven patients with various gastrointestinal carcinomas and one patient with cancer of the prostate during a phase I clinical trial with combined FT and radiation treatment conducted by the Radiation Oncology Departments of the Veterans Administration Medical Center and the University of California Medical Center. These patients were monitored during 14 cycles of treatment. Each cycle of treatment consisted of daily 1.0-g/m² infusions of FT (5–10 minutes) for 5 days and daily 250-rad radiation doses starting on the second day for 4 days. Each cycle was followed by 9 days of rest before the next treatment cycle. With the exception of one sample drawn 4 hours after the daily FT dose, blood was always drawn 24 hours after each FT dose and immediately before the succeeding dose. The serum was separated and frozen at –20°C until analysis.

The analysis of serum 5-FU, FT, and DFT was performed on a Hewlett-Packard (Avondale, PA) 1084B microprocessor-controlled high-performance liquid chromatograph (HPLC) equipped with a variable wavelength detector, automatic sampling system, and a Waters Associates (Milford, MA) 4 mm × 30-cm μBondapak C_{18} column. The column was run at ambient temperatures at a flow rate of 2 ml/minute. The elution solvent was 5% methanol in 0.01 M aqueous sodium acetate (pH 4.0) yielding retention times of approximately 2.5, 21.5, and 25 minutes for 5-FU, FT, and DFT, respectively. The variable UV detector was set at a sample wavelength of 270 nm and a reference wavelength of 330 nm. Samples were extracted by adding 0.5 ml of water, 0.1 ml of 0.5 M monobasic sodium phosphate, and 8 ml of ethyl acetate to 0.5 ml of plasma (10). Separate 5-FU and FT standards (DFT was compared to the FT standards) were run for each assay by adding various concentrations of the two drugs to sera. The ethyl acetate was removed after centrifugation and evaporated to dryness under N_2 at 50°C. The residue was taken up in 100 μl of methanol, and 20 μl was injected into the HPLC. When filtration was used, serum was passed through Amicon centriflow membrane filters (type CF50A) before extraction and chromatography as above. Serum volume was doubled since approximately one half of the serum does not pass through the filter during centrifugation.

Gas chromatographic/mass spectrometric (GC/MS) analysis of 5-FU from serum of patients infused with 5-FU was performed on a Finnigan (Sunnyvale, CA) 4000 GC/MS with a 6110 data system using chemical ionization and selected ion monitoring. The 6-foot gas chromatography column was packed with 3% SP2250 on 100/120 mesh Supelcoport (Supelco, Bellefonte, PA). The injector, column oven, and transfer lines were maintained at 250°C, 150°C, and 250°C, respectively. Methane served as both the carrier gas and chemical ionization reagent gas at a flow of 10 ml/minute. Ions at 271 and 275 mass/charge (m/e) were monitored for the internal standard thymine and 5-FU, respectively. Samples were extracted by adding 2 ml of saturated ammonium sulfate solution, 35 μl of 0.5 M sodium acetate, and 10 ml of 20% isopropanol in ethyl ether to 0.5 ml of plasma to which 200 ng/ml of thymine had been added previously. The extraction solvent was centrifuged, removed, and evaporated to dryness under N_2 at 60°C, and the residue was taken up in 40 μl of 50% *N,O*-bis-(trimethylsilyl)-trifluoroacetamide in pyridine. After warming this mixture at 60°C for 15 minutes, 1 μl aliquots were injected into the GC/MS.

For analysis of DFT as 5-FU, the DFT peaks from various HPLC runs were collected free of any detectable FT and allowed to sit in the dark at room temperature overnight. On the next day, the individual DFT fractions were extracted with eight volumes of ethyl acetate, evaporated to dryness, and analyzed by GC/MS for 5-FU and by HPLC for both DFT and 5-FU as described above. Structural analysis of DFT was performed on the GC/MS solid probe using ammonia chemical ionization. The probe was set ballistically to increase from ambient temperature at 100°C/minute while the mass spectrometer scanned from 50 to 250 m/e at a rate of 2 msec/mass. Source pressure and temperature were set at 0.07 torr and 220°C, respectively. The DFT sample was isolated from 2 ml of patient serum using HPLC as described above. The DFT peaks were pooled, immediately re-extracted with eight volumes of ethyl acetate, and evaporated to dryness. The residue was taken up in ≈ 15 μl of methanol for transfer to a solid-probe glass capillary tube and re-evaporated to dryness for direct insertion into the mass spectrometer.

RESULTS

No concentrations of 5-FU > 25 ng/ml were detected by HPLC in any of the 43 serum specimens taken at 24 hours after daily 1.0-g/m² bolus FT doses, although there appeared to be trace amounts in several sera. In addition, a serum sample taken

TABLE 1.—Average FT and DFT serum concentrations and average relationship between FT and DFT concentration

Patient No.	No. of cycles*	No. of sera tested	FT (μg/ml)	DFT (μg/ml)	FT/DFT
1	1	3	18	0.98	18
2	1	3	9.8	0.65	15
3	2	8	12	0.54	22
4	1	4	19	1.0	19
5	1	4	17	0.47	36
6	2	6	24	0.80	30
7	2	6	6.8	0.31	22
8	4	9	20	1.2	17

*For each cycle, levels of FT and DFT were measured in 2–4 serum samples.

4 hours after the FT dose from one of these patients showed no measurable level of 5-FU. In order to demonstrate positive systemic 5-FU serum levels with the assay, sera were measured from two other patients who had been infused with 5-FU. These patients were infused at a rate of 25 mg/kg of body weight/24 hours (≈ 1.0 g/m²), and serum samples were acquired 24 hours after infusion was started. The 5-FU levels measured in these sera by our HPLC method were 410 and 550 ng/ml and compared closely with the concentrations (440 and 520 ng/ml, respectively) determined by GC/MS using chemical ionization and selected ion monitoring.

Other published results have indicated highly elevated serum 5-FU at 24 hours (2 μg/ml) following a high FT dose of 5 g/m² (6). These results were obtained by passing the patient serum through filters that exclude materials > 50,000 daltons before running the unextracted filtrate similarly by HPLC. In order to determine if the filtration procedure would give substantially different results, samples from three patients given FT were divided into two aliquots each and treated with and without filtration. The three unfiltered and three filtered aliquots were subsequently extracted and chromatographed as before. The unfiltered samples had 5-FU values < 25 ng/ml (but not negative) while the filtered samples had 5-FU values only slightly higher, ranging from 25 to 40 ng/ml. There was very little variation of the FT and DFT concentrations between the filtered and unfiltered sera.

In addition to FT, every patient serum appeared to contain a major FT metabolite (DFT) eluting from HPLC after FT. In a recent study of the metabolism of FT in man, work done by electron-impact mass spectrometry strongly indicated the presence in serum and urine of a metabolite (MH-4) differing from FT by only a double bond in the furan ring (8). In a preliminary attempt to prove DFT to be the same metabolite and confirm the proposed structure, we isolated this compound from FT by HPLC and analyzed it by solid-probe mass spectrometry using ammonia chemical ionization. The two largest peaks in the mass spectrum of the compound (which began to boil off the probe at about 80°C) constituted > 20% of the total ion current and were at 199 (11%) and 216 (12%) m/e. The peak at 199 m/e probably corresponds to the protonated molecular ion of the proposed DFT structure, and the base ion at 216 m/e would then correspond to the attachment of NH_4^+ to the intact molecule. No other peaks in this spectrum constituted > 5% of the total ion current. There were peaks at 68 (3%), 69 (1%), and 131 (5%) m/e, which could correspond to fragmentation of DFT (8), but sample and/or source contamination made interpretation of these peaks unreliable.

To substantiate the DFT peak as a metabolite of FT, the labile DFT peaks were collected from HPLC runs of several patient sera, treated as previously described, and then analyzed by both HPLC and GC/MS. The HPLC scans revealed no remaining DFT and conversion to 5-FU. The presence of 5-FU was further substantiated by GC/MS analysis. As it is not convenient to isolate and characterize every DFT peak in this manner, the presence of this metabolite in each patient sample was routinely determined by the ratio of respective retention times of FT over DFT. This ratio varied very little throughout the study (1.15–1.18), and as monitored by HPLC at 270 nm, neither the standard sera with added FT nor the sera from patients given 5-FU contained interference peaks eluting in this retention time frame.

Table 1 illustrates the interpatient variation in FT and DFT serum concentrations at 24 hours after the daily 1.0-g/m² dose. Serum concentrations for all eight patients averaged 16 and 0.74 μg/ml for FT and DFT, respectively, during the 14 cycles of treatment. The range for the 43 sera tested was from 5 to 29 μg/ml for FT. In six of the eight patients monitored, there appeared to be a close relationship

between daily 24-hour serum concentrations of FT and the DFT metabolite (FT/DFT), ranging from 15/1 to 22/1. In the remaining two patients, this ratio was clearly higher at 30/1 and 36/1.

DISCUSSION

The experiment designed to test for in vitro 5-FU by using filtration before extraction indicated only a small increase in 5-FU and is not sufficient to explain the elevated serum 5-FU seen with FT doses that were two to five times higher than in the present study (6,7). Under the conditions of our study with concurrent FT chemotherapy and radiotherapy, systemic serum 5-FU concentrations are very low at 24 hours (> 25 ng/ml) compared with the much higher serum concentrations of 5-FU found in our 5-FU infusion-radiation regimens known to be therapeutic (2,4,11). Since the clinical side effects and response rates of FT very closely resemble those seen after 5-day infusions of 5-FU, it seems likely that FT is intracellularly converted to 5-FU. Some data from rodent studies support this interpretation (12,13). However, it is clear that extracellular levels of 5-FU known to be associated with radiosensitization (≥ 400 ng/ml) (14) are not obtained by conventional daily FT doses. Since animal studies have shown that repeated administration of 5-FU but not FT leads to enhanced serum 5-FU levels (15), we feel that prior reports of very high 5-FU levels obtained by high-dose FT injections are unlikely to be helpful.

Based on studies with both HPLC and GC/MS, we conclude that the DFT monitored in this study and the MH-4 metabolite reported earlier (8,9) are the same metabolite of FT. Further, evidence of a protonated molecular ion from solid-probe mass spectrometry of the DFT metabolite supports the structure proposed for MH-4. The observed high concentration and lipophilicity of DFT compared to 5-FU appear to make this metabolite a more immediate candidate for study in serum. The significance of the change in ratio of the serum concentrations of FT and DFT in two of our patients is not known. It remains an intriguing possibility that variations in DFT levels with respect to FT may have some relationship to side effects (16), especially nausea and vomiting, which have been highly variable among patients in our experience.

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High-Dose Cisplatin Therapy Using Mannitol Versus Furosemide Diuresis: Comparative Pharmacokinetics and Toxicity[1]

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SUMMARY

The dose-limiting toxic effect of high-dose (100 mg/m²) cisplatin is renal insufficiency. Hydration with furosemide- or mannitol-induced diuresis has been reported to ameliorate this toxicity. Animal studies suggest that mannitol may be superior to furosemide in this regard. Twenty-two patients with advanced neoplasms refractory to conventional therapy were treated with cisplatin at a dose of 100 mg/m² every 21–28 days. Patients were randomized to receive 37.5 g of mannitol by 6-hour infusion with cisplatin or 40 mg of furosemide prior to cisplatin therapy. Hydration with at least 1 liter of normal saline was given prior to cisplatin. Nephrotoxicity (creatinine > 2 mg/100 ml, creatinine clearance < 50 ml/minute) occurred in 19% of courses in the furosemide-treated group and in 28% of courses in the mannitol-treated group. Peak plasma platinum concentration, terminal half-life, urinary excretion, and percent protein-bound plasma platinum were similar in both groups. The use of cisplatin at this dose schedule resulted in similar toxicity and pharmacokinetics when using hydration with either furosemide or mannitol.

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Cisplatin, a heavy metal drug with antitumor properties, has activity against a number of solid tumors, including germinal neoplasms of the testes, bladder cancer, ovarian cancer, and squamous cell cancer of the head and neck (5,12,22,24,25,27). Evidence of activity in thyroid cancer, osteosarcoma, and carcinoma of the cervix has also been demonstrated (17,20,23).

Toxic effects associated with cisplatin therapy include gastrointestinal disturbances, neuropathy, myelosuppression, and ototoxic effects. However, the major dose-limiting toxic effect appears to be renal insufficiency (16). Cisplatin is known to cause renal tubular damage (11,16). Vigorous hydration and diuresis with mannitol or furosemide have ameliorated this toxicity (10,14,17,24,26). The exact mechanism of diuretic-induced reduction of nephrotoxicity is not known. Postulated mechanisms include accelerating the passage of cisplatin through renal tubules by increased urinary excretion, reversing the osmotic gradient in the tubules by mannitol, and blocking sodium and water reabsorption by furosemide. Mannitol may exert a protective effect by binding to cisplatin as demonstrated in vitro (6). Studies in rats indicate that mannitol, but not furosemide, may be protective against high doses of cisplatin (19).

We undertook a randomized prospective trial of high-dose cisplatin and hydration with mannitol- or furosemide-induced diuresis in order to evaluate which, if either, diuretic is superior in reducing the frequency of renal insufficiency and to compare the

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pharmacokinetics of cisplatin when either diuretic is administered.

METHODS

Twenty-two patients aged 18–69 years with histologically documented neoplasms refractory to conventional therapy were admitted to the study between March 1, 1978, and February 1, 1979. Patients were eligible if they were able to tolerate a fluid load and had serum creatinine < 1.5 mg/100 ml and creatinine clearance > 50 ml/minute.

All patients gave informed consent. Baseline studies included complete history and physical examination, complete hemogram, serum electrolyte levels, liver function tests, and 24-hour urine for creatinine and protein. For 4 hours prior to cisplatin therapy, 1500 ml of 0.154 M NaCl with 20 mEq of KCl were given by iv infusion. Cisplatin was given at a dose of 100 mg/m² in 2 liters of 5 g/100 ml of dextrose and 0.077 M NaCl by 6-hour iv infusion. Following cisplatin therapy, at least 1 liter of fluid (usually 0.154 M NaCl) was given by continuous iv infusion. Diuresis was achieved with 37.5 g of mannitol in 375 ml given concurrently with the 6-hour cisplatin infusion or with a 40-mg iv bolus of furosemide given within 60 minutes before the start of cisplatin infusion. Randomization of computer-generated cards for mannitol versus furosemide diuresis was performed before therapy began.

Blood samples were collected in heparinized tubes and centrifuged at 1000 × *g* for 10 minutes, and the plasma was removed and frozen until analyzed. Protein-free plasma ultrafiltrate was prepared by centrifuging 3 ml of freshly obtained plasma in a Centriflo CF50 membrane (Amicon Corp, Lexington, MA) at 1000 × *g* for 20 minutes at 4°C. Urine samples were collected as voided, measured, and stored at –40°C either individually or as pooled 6- or 24-hour collections. Plasma and urine samples were analyzed for platinum by flameless atomic absorption spectrometry (15).

RESULTS

Ten patients received mannitol diuresis during cisplatin therapy. A total of 22 courses of cisplatin were given (median, two courses; range, one to four). There were nine females and one male, with a median age of 51 years (range, 31–69). Seven patients had carcinoma of the breast and three had soft tissue sarcoma.

Twelve patients received furosemide and cisplatin. A total of 25 courses were given (median, two courses; range, one to four). There were eight females and four males. The median age was 48 years (range, 18–54). Four patients had carcinoma of the breast, three had soft tissue sarcoma, two had carcinoma of the lung, and one each had ovarian cancer, colon cancer, and Hodgkin's disease.

No patient in either group received another known nephrotoxic agent (ie, aminoglycoside antibiotics) while receiving cisplatin therapy. Prior to initiation of cisplatin therapy, all patients had creatinine clearance > 50 ml/minute and most had clearances > 70 ml/minute, with normal urinalysis and 24-hour urinary protein excretion.

Toxic Effects

Nephrotoxicity (creatinine clearance < 50 ml/minute and serum creatinine > 2 mg/100 ml) occurred in 19% and 28% of courses in patients treated with cisplatin and either furosemide or mannitol diuresis, respectively. The median increase of serum creatinine per course was similar in the mannitol-treated patients (0.4 mg/100 ml) as compared to the furosemide-treated patients (0.3 mg/100 ml). The median decrease in creatinine clearance for mannitol-treated patients (34 ml/minute) compared to furosemide-treated patients (26 ml/minute) was also similar. A t test for mean values (4) was used to analyze the mean changes in serum creatinine levels and creatinine clearance before and after drug administration. The highest serum creatinine levels measured after each course were compared to the pretherapy levels. The lowest creatinine clearance obtained per patient during therapy was compared to the pretherapy value. No significant differences were found between mannitol- and furosemide-treated patients with regard to serum creatinine levels ($P > 0.45$) or creatinine clearance ($P > 0.25$). In two patients receiving cisplatin and mannitol, persistent renal insufficiency necessitated stopping therapy after one and four courses, respectively. In two other patients who developed progressive disease while receiving cisplatin and mannitol and in whom cisplatin was stopped, plasma creatinine persisted at > 2 mg/100 ml. One patient had received two courses of cisplatin, the other patient, three courses. Hydronephrosis, demonstrated by abdominal sonogram, was noted in one of these patients after cessation of cisplatin therapy. There was no evidence of hydronephrosis prior to cisplatin therapy. Renal insufficiency in this patient may have been secondary to both cisplatin therapy and ureteral obstruction by tumor. In one patient who received cisplatin and furosemide, partial ureteral obstruction documented by iv pyelogram was observed following therapy. The patient developed

transient (< 1 month's duration) nephrotoxic effects after cisplatin therapy. In this case the nephrotoxicity was thought to be secondary to cisplatin therapy because it was transient despite evidence of progressive disease. No other patient in either group had any evidence of ureteral obstruction by tumor either prior to or following cisplatin therapy.

Pharmacokinetics

Nine patients receiving cisplatin and furosemide and seven patients receiving cisplatin and mannitol had pharmacokinetic studies performed during one or more courses of therapy. Plasma platinum pharmacokinetics were similar in patients diuresed with mannitol or furosemide (fig 1). In both groups of patients, there was a gradual increase in plasma platinum concentrations during the 6-hour infusion of cisplatin. The peak plasma platinum concentrations achieved were similar in mannitol (4.06 ± 0.41 μg/ml) and furosemide (3.53 ± 0.77 μg/ml) groups and agree with previous reports (3). After completion of the cisplatin infusion, there was a decrease in plasma platinum, with no difference between furosemide- and mannitol-diuresed groups. This post-infusion decline in plasma platinum concentrations was far more complex than previously reported. Specifically, an initial rapid decrease in plasma platinum was followed by a rebound increase at 18–24 hours and by a slow terminal phase of platinum elimination (fig 1). The terminal plasma half-life of platinum was ≈ 190 hours. Platinum in the plasma was rapidly bound to protein in both mannitol- and furosemide-treated patients (fig 2). The percent of plasma platinum that was filterable decreased even during the cisplatin infusion. Once the input of cisplatin ceased, the percent of free platinum decreased rapidly with a half-life of ≈ 25 minutes, so that by 8 hours the amount of filterable platinum was below the detection limits of flameless atomic absorption spectrometry.

The urinary excretion of platinum was similar in both furosemide- and mannitol-diuresed patients (table 1). In both groups, the greatest excretion of platinum occurred during the first 6 hours of therapy. In certain individuals, urinary platinum excretions were determined for several days. In five patients, the percent of administered dose excreted from 24 to 48 hours was 2.3% ± 0.6% and from 48 to 72 hours was 1.8% ± 0.8%. From 96 to 192 hours, the daily excretion of platinum was approximately 1% of the dose administered.

In two patients diuresed with mannitol and in two diuresed with furosemide, individual urine specimens were analyzed for platinum. Semilogarithmic

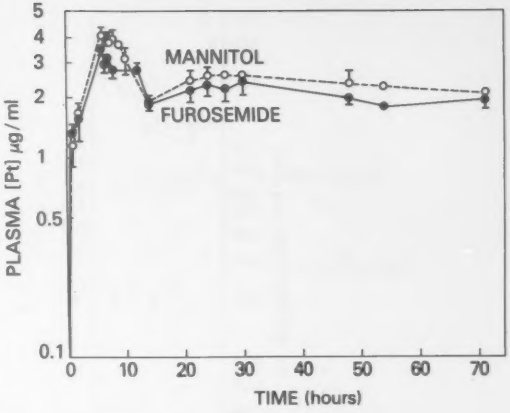

FIGURE 1.—Mean (± SEM) plasma platinum concentrations in cisplatin-treated patients diuresed with mannitol (O- - - -O; 7 patients) or furosemide (●——●; 9 patients). Plasma platinum concentrations were determined by atomic absorption spectrometry as described in Methods section.

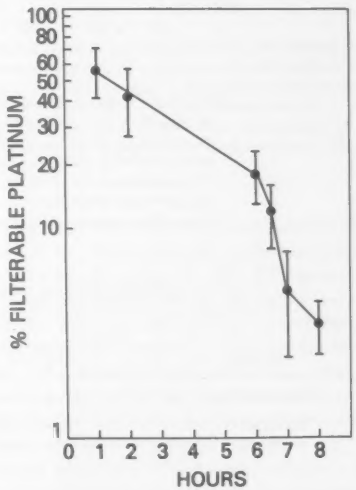

FIGURE 2.—Mean (± SD) filterable platinum species in plasma of 5 patients. Plasma samples were obtained at various times after cisplatin administration, and 3-ml aliquots were centrifuged at 1000 × *g* for 20 mins in an Amicon Centriflo membrane as described in Methods section. Platinum concentrations in whole plasma and protein-free ultrafiltrate were determined by flameless atomic absorption spectrometry.

plots of the rate of urinary platinum excretion versus time were biphasic (fig 3), allowing kinetic analysis by a two-compartment model (8). The results of these analyses are shown in table 2. For

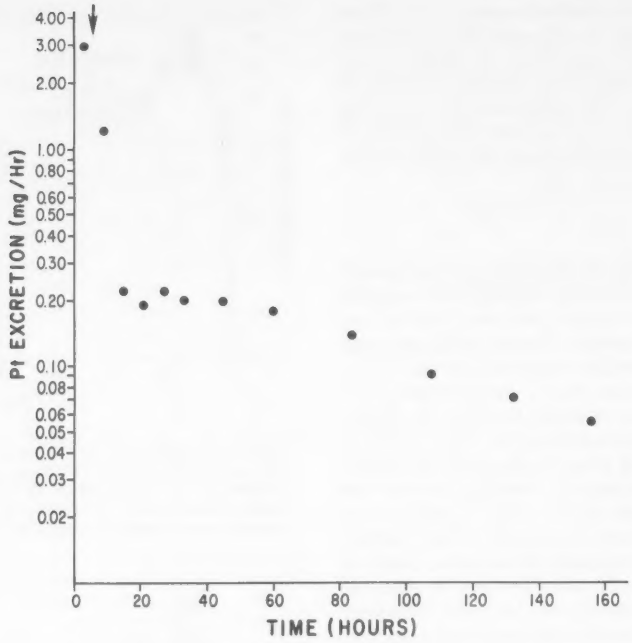

FIGURE 3.—Urinary excretion of platinum from 1 patient. Urinary concentration of platinum was determined by flameless atomic absorption spectrometry as described in Methods section. Total platinum in each sample was calculated using platinum concentrations and sample volume. Rate of platinum excretion was calculated by dividing total platinum excreted by the time elapsed from the previous urine sample. Points are plotted at the half-time between successive urine samples. The arrow denotes cessation of cisplatin infusion.

TABLE 1.—Urinary platinum excretion by patients diuresed with furosemide or mannitol

Diuretic	% administered dose excreted (mean ± SE) in—				
	0–6 hrs	6–12 hrs	12–18 hrs	18–24 hrs	0–24 hrs
Furosemide	11.4 ± 2.4	3.4 ± 1.2	0.8 ± 0.3	1.9 ± 0.8	17.5 ± 2.9
Mannitol	9.4 ± 0.9	3.4 ± 0.8	0.6 ± 0.2	0.7 ± 0.2	14.2 ± 0.9

each patient there was good agreement between the cisplatin terminal half-life calculated from urinary excretion and that actually measured in plasma.

Two patients in this study had pleural effusions at the time of cisplatin therapy. In one of these patients, a number of simultaneous effusion and plasma samples were obtained and platinum concentrations determined (fig 4). As shown in fig 4, at the end of cisplatin infusion, there was a significant concentration of platinum in the pleural effusion, although it was substantially less than that in the plasma. However, the removal of platinum from the plasma was much more rapid (half-life = 204 hours) than from the effusion (half-life = 535 hours) so that by 10 days, the effusion platinum concentration exceeded that of the plasma. In the second patient, the effusion platinum concentration at 24 hours was 28% of the concomitant plasma concentration.

DISCUSSION

Neither mannitol nor furosemide appeared superior to the other in reducing the frequency of

nephrotoxicity in our group of patients. Of the four patients receiving mannitol who had persistent nephrotoxic reactions following cessation of therapy, three had severe nausea and vomiting during therapy and one had possible disease-related renal involvement. Three of the four patients had received prior cisplatin courses without persistent nephrotoxic effects. The fact that all four of the patients received mannitol and that none receiving furosemide developed persistent nephrotoxic effects indicates that further comparisons are necessary. It has been suggested that hydration alone can prevent cisplatin-induced nephrotoxicity. Two recent prospective studies comparing hydration with and without mannitol diuresis have been reported (1,2). In one study using the same dose of cisplatin (100 mg/m²) as in our study, mannitol ameliorated nephrotoxicity better than hydration alone. In the other study using the same dose, there was no difference between groups receiving mannitol and hydration or hydration alone. Pharmacokinetic information was not reported in either study.

We observed no differences in platinum pharmacokinetics of patients diuresed with mannitol or furosemide. Mannitol- and furosemide-treated patients had similar peak plasma platinum concentration, terminal half-life, protein binding, and urinary excretion. The peak plasma platinum concentrations and percent of administered dose excreted in the first 24 hours are consistent with previous reports (3). Filterable cisplatin, thought by some to possibly be the active species (22), was equal in both mannitol- and furosemide-treated groups and decreased during the 6-hour infusion. The half-life of filterable cisplatin and the percent that was filterable agreed with reports of other investigators (18). Whether the protein-bound fraction, with its long terminal half-life, releases an "active species" following the infusion of cisplatin remains to be determined. The plasma terminal half-life determined in our studies agrees with that reported by Smith and Taylor (21) but is much longer than that reported by others (3,7,9). This is most likely explained by our analysis of samples obtained at later times after drug administration, whereas studies defining shorter terminal half-lives were based on samples collected over much shorter periods of time (3,7,9). Our determination of the terminal half-life is probably more accurate than the shorter times previously reported. Gormley and co-workers (9) reported a half-life of 67 hours but stated that calculations using this value grossly underestimated the plasma platinum concentrations actually measured 21 days after therapy. This implies that the actual terminal half-life is significantly longer than 67 hours. Frick et al (7) report terminal cisplatin

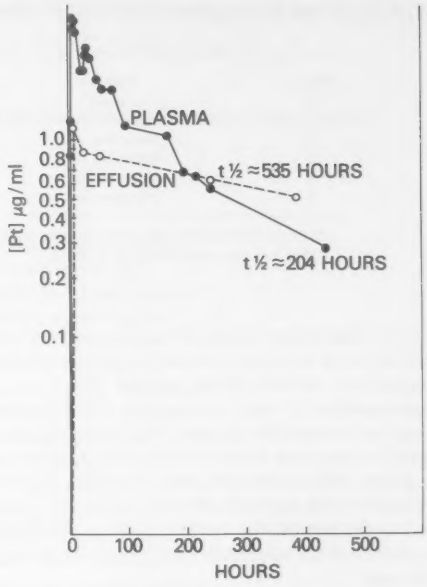

FIGURE 4.—Platinum concentrations in the plasma and malignant pleural effusion of a cisplatin-treated patient with carcinoma of the breast. At various times after drug administration, samples of plasma (●——●) and pleural fluid (○- - -○) were obtained, and platinum content was determined by flameless atomic absorption spectrometry as described in Methods section.

half-lives of 15.0–57.7 hours, based solely on urinary excretion data. This is almost certainly due to analysis of samples obtained to only 30 hours after cisplatin administration. When we analyzed our data in a similar fashion, but utilized samples obtained for a minimum of 48 hours after initiation of cisplatin therapy, we determined half-lives that agreed closely with the terminal plasma platinum half-life actually found for each patient. Moreover, if we based our analysis on urine samples obtained for 0–30 hours only, we estimated half-lives of 32–44 hours, which again agreed with the actual plasma decline in platinum concentrations. However, the plasma pharmacokinetics of cisplatin from 24 to 30 hours clearly do not represent the terminal half-life.

The use of high-dose cisplatin given in this dose and schedule has demonstrated that nephrotoxicity may occur despite hydration and mannitol- or furosemide-induced diuresis. This toxicity necessitated discontinuance of cisplatin in two patients and persisted in four patients. Neither diuretic appeared superior to the other in ameliorating nephrotoxicity. The use of hydration and diuretics to establish a large urine volume in a relatively short

TABLE 2.—Cisplatin terminal half-life as calculated from urinary excretion and actually measured in plasma

Patient No.	Diuretic used	β*(hr^{-1})	Plasma half-life† of cisplatin (hr^{-1})	
			Calculated	Actual
1	Mannitol	0.0064	107	108
2	Mannitol	0.0018	169	192.5
3	Furosemide	0.0033	208	204
4	Furosemide	0.0033	208	210

*β = apparent rate constant for the terminal phase.
†Half-life was calculated as 0.693/β.

amount of time may be an advantage compared to hydration alone in the treatment of patients receiving cisplatin in an outpatient setting (13).

Pharmacokinetic data were similar both in mannitol and in furosemide groups. Further studies are indicated to measure tissue distribution of platinum when given with hydration and diuretics. The use of hydration with diuresis should be further investigated to determine the optimal way to ameliorate the nephrotoxicity associated with high-dose cisplatin therapy.

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Cardiomyopathy and Other Chronic Toxic Effects Induced in Rabbits by Doxorubicin and Possible Prevention by Coenzyme Q_{10}[1]

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SUMMARY

Cumulative dose-dependent toxic effects, particularly cardiomyopathy, induced by doxorubicin and the possible prevention by coenzyme Q_{10} (CoQ_{10}) were studied in rabbits. In rabbits given doxorubicin alone, there was considerable body weight loss, alopecia, pancytopenia, significant increase in serum creatine phosphokinase and LDH, and ECG changes characterized by tachycardia, flat and inverted T wave, and premature ventricular contractions. In rabbits given doxorubicin plus CoQ_{10}, the only change was pancytopenia. In rabbits treated with doxorubicin alone, the most prominent histologic changes were observed in mitochondria of myocytes, and these changes were characterized by loss of outer membrane, disarrangement of cristae, and formation of numerous concentric lamellae. In addition to mitochondrial changes, there were numerous vacuolizations and extensive depositions of both electron-dense and membranous laminated bodies in the sarcoplasm and disarrangement of Z-band and filamentous changes of myofibrils. Numerous vacuolizations in the capillary endothelial cytoplasm in the myocardium were also conspicuous. On the other hand, few significant morphologic changes were seen in the nuclei of myocytes. There were few ECG and histopathologic changes in rabbits treated with both doxorubicin and CoQ_{10}. These findings suggest that the cardiomyopathy of doxorubicin may be prevented or is at least inhibited by CoQ_{10}. The mechanisms of both doxorubicin-induced cardiomyopathy and its prevention by CoQ_{10} are discussed.

[Cancer Treat Rep 65:79–91, 1981]

Doxorubicin is an antitumor antibiotic of the anthracycline group isolated in 1967 at the Farmitalia Research Laboratories (Milan, Italy) from cultures of *Streptomyces peucetius* var. *caesius* (1). It is well-known that doxorubicin has produced encouraging clinical responses in the chemotherapeutic treatment of malignant lymphomas (2–4), leukemias (2,3,5,6), and some solid tumors (2,3,6,7).

In addition to its promising chemotherapeutic potential, doxorubicin has undesirable side effects which include cardiomyopathy, severe bone marrow suppression, alopecia, and oral ulcerations (2,3,8). In particular, doxorubicin induces cardiomyopathy and an irreversible heart failure which are resistant to digitalis. These major factors limit the establishment of maximal recommended total drug doses (9–11).

In anthracycline antibiotics, rings B and C are

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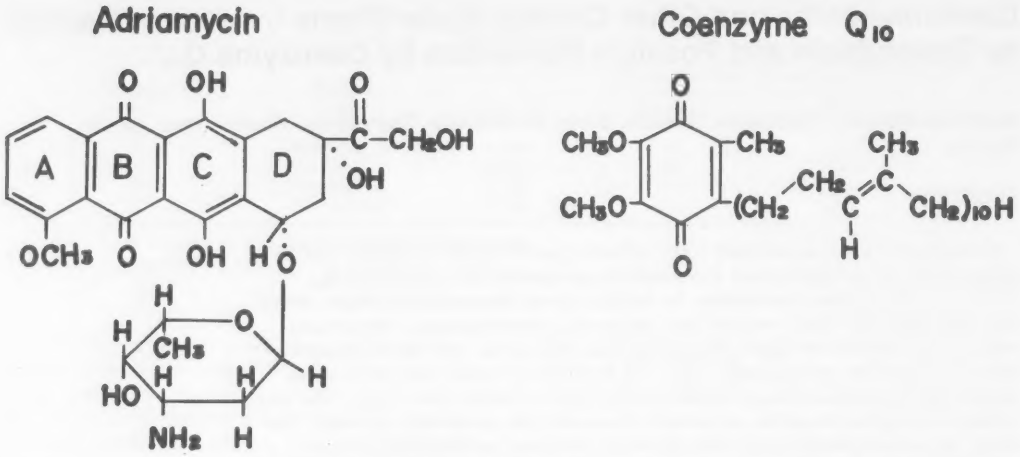


FIGURE 1.—Quinone relationships of doxorubicin and CoQ_{10} in structural formula. In doxorubicin, rings B and C are shown as quinoid and hydroquinoid.

shown as quinoid and hydroquinoid in the structural formula (fig 1), and a possible inhibiting action of coenzyme Q_{10} (CoQ_{10}) enzymes such as succinoxidase and NADH-oxidase in mitochondria has been suggested (12,13).

We attempted to produce a rabbit model with an induced cardiomyopathy similar to that observed in cancer patients after prolonged chemotherapy with doxorubicin. Using this model we studied the side effects produced by doxorubicin and the potential preventive effect produced by CoQ_{10} (14–17).

MATERIALS AND METHODS

Experimental Animals and Chemotherapeutic Agents

Sixteen male New Zealand white rabbits, initially weighing 2.5 kg, were separated into four groups (A, B, C, and D). Six rabbits in group A were given 1.0 mg(0.2 ml)/kg/day of doxorubicin (Kyowa Hakko Kogyo Co, Ltd, Tokyo, Japan). Six rabbits in group B were separately given 1.0 mg(0.2 ml)/kg/day of doxorubicin and 2.5 mg(0.25 ml)/kg/day of CoQ_{10}. Each chemotherapeutic agent was given iv alone or in combination to all animals (disposable 1.0-mg syringe, Terumo Co, Ltd, Japan) three times per week. Two rabbits in group C were given 2.5 mg(0.25 ml)/kg/day of CoQ_{10} and two rabbits in group D were given only the solvent of CoQ_{10} (0.5 ml), as controls (Eisai Co, Ltd, Tokyo, Japan).

Experimental Design

General conditions were monitored by body weight changes, and evidence of bone marrow suppression was obtained by periodic routine hematologic examinations (erythrocyte, hemoglobin, leukocyte, and platelet determinations). Serum enzymes were determined serially throughout the experimental courses to obtain indications of myocardial cellular damage. Both SGOT and SGPT were determined by the Reitman-Frankel method (18) and were expressed in Karmen units. LDH was measured by the Babson-Phillips method (19) and was expressed in Wroblewski units. CPK was determined by the Rosalki method (20) and was expressed in milliunits per milliliter.

An ECG was recorded before starting treatment and thereafter once a month, using 50- or 100-mm/sec paper speed and the apparatus of the Electronics for Medicine.

After treatments with doxorubicin at cumulative doses of 25 mg/kg, the rabbits were killed as soon as possible following administration of pentobarbital sodium anesthesia and O_2 inhalation. The heart was excised in toto, and sections from the left ventricular free wall were immediately cut into about 1.0-mm^3 cubes in 2.5% glutaraldehyde solution in 0.05 M phosphate-buffered saline (PBS), pH 7.4, at 4°C. In control experiments, histologic and/or ultrastructural damages in the myocardium were absent when these conditions were employed. These specimens were subsequently fixed in fresh-buf-

fered glutaraldehyde for 2 hours at 4°C. After being washed in 0.05 M PBS containing 0.1 M sucrose, pH 7.4, five times at 4°C, these specimens were post-fixed in 1% OsO_4 in 0.1 M PBS, pH 7.4, for 2 hours at 4°C; dehydrated through ascending concentrations of ethanols; transferred to propylene oxide; and, finally, embedded in EPON (TAAB Lab, England). Specific samples, consituted of longitudinally oriented myocardial fibers, were chosen under the light microscope from semi-thin sections stained with 0.5% toluidine blue. Subsequently, the ultra-thin sections obtained by cutting with glass knives were stained with uranyl acetate and lead citrate and then examined under a Hitachi HU-11D electron microscope.

RESULTS

Clinical Signs

Serial body weight measurement revealed that rabbits given doxorubicin alone (group A) gained weight at a rate similar to that of animals given both doxorubicin (10 mg/kg) and CoQ_{10} (group B). Thereafter, rabbits in group A failed to maintain weight gains, and a rapid drop in body weight occurred when a dose > 20 mg/kg of doxorubicin was given. On the other hand, rabbits given both doxorubicin and CoQ_{10} continued to gain weight, even when the cumulative dose of doxorubicin was > 20 mg/kg. Prominent alopecia did not occur in rabbits given doxorubicin and CoQ_{10} (group B) but did occur in rabbits given more than the cumulative dose of 15 mg/kg of doxorubicin (group A) (data not shown).

Figure 2 shows the hematologic findings expressed as mean actual value per rabbit. Pancytopenia, particularly leukopenia and thrombocytopenia, occurred in both groups A and B, indicating that simultaneous administration of CoQ_{10} did not prevent the pancytopenia induced by doxorubicin. Both SGOT and SGPT were slightly elevated in group A rabbits. Both serum CPK and LDH levels significantly increased in group A rabbits by the end of this experiment. On the other hand, in rabbits given doxorubicin and CoQ_{10} (group B), these enzyme levels remained unchanged throughout the period of observation (fig 3).

The ECG abnormalities induced by doxorubicin included supraventricular tachycardia, premature ventricular contractions, ST segment and T-wave changes, and low voltage (fig 4A). However, ECG in rabbits given doxorubicin and CoQ_{10} (group B) showed little change except in one rabbit in which inverted T wave was apparent (fig 4B).

Histopathologic Examinations

Surviving rabbits in each group were killed for histologic examination after the cumulative dose of doxorubicin had reached 25 mg/kg, since in a preliminary pilot study, none of the rabbits given > 25 mg/kg of doxorubicin as a cumulative dose survived.

Gross examination of the heart from group A rabbits at the cumulative dose of 25 mg/kg of doxorubicin revealed pale foci in both ventricular free walls and in the interventricular septum as well as profuse pericardial effusion. In contrast, neither pericardial effusion nor pale foci were observed in group B rabbits. In group A rabbits, the most prominent ultrastructural changes were observed in mitochondria of the left ventricular myocardium (fig 5). The mitochondrial alterations were characterized by disarrangement of cristae, focal dilations or loss of the outer mitochondrial envelope, and formation of numerous concentric membranous lamellae, including electron-dense bodies in the mitochondria and alteration to an electron-dense matrix (fig 5A). In addition, severely degenerated mitochondria containing many inclusion bodies were observed (fig 5B). Accompanying those mitochondrial alterations was a progressive disorganization of the myocardial contractile elements, characterized by swelling of the sarcoplasmic reticulum, varying degrees of sarcoplasmic vacuolization (fig 5C), loss of registry of Z-bands (fig 5A and C), and loss of a characteristic parallel orientation of myofibrils. Some myofibrils were fragmented, and there was a progressive disappearance of organized bundles of actin and myosin filaments (fig 5D). Severe degenerative change was characterized by extensive depositions of both concentric membranous laminated bodies and electron-dense bodies within the sarcoplasm (fig 5E). In general, nuclear alterations were not striking in degenerating myocardial cells, although there was nucleolar segregation in some myocardial cells (fig 5F). Another conspicuous change in the rabbits given doxorubicin alone was the numerous vacuolizations in the capillary endothelial cytoplasm in the myocardium (fig 5G), suggesting that the vascular systems in the myocardium are also directly damaged with administration of doxorubicin. On the other hand, these histologic changes observed in rabbits treated with doxorubicin alone were rarely found in the rabbits treated concomitantly with doxorubicin and CoQ_{10} (fig 5H and I). Slight disarrangement of mitochondrial cristae, forming both concentric membranous lamellae (fig 5J), the electron-dense inclusion body in mitochondria (fig 5K), and a few vacuolizations in the sarcoplasm were observed only in a limited area in the myocardium of the group B rabbits. These findings suggest that

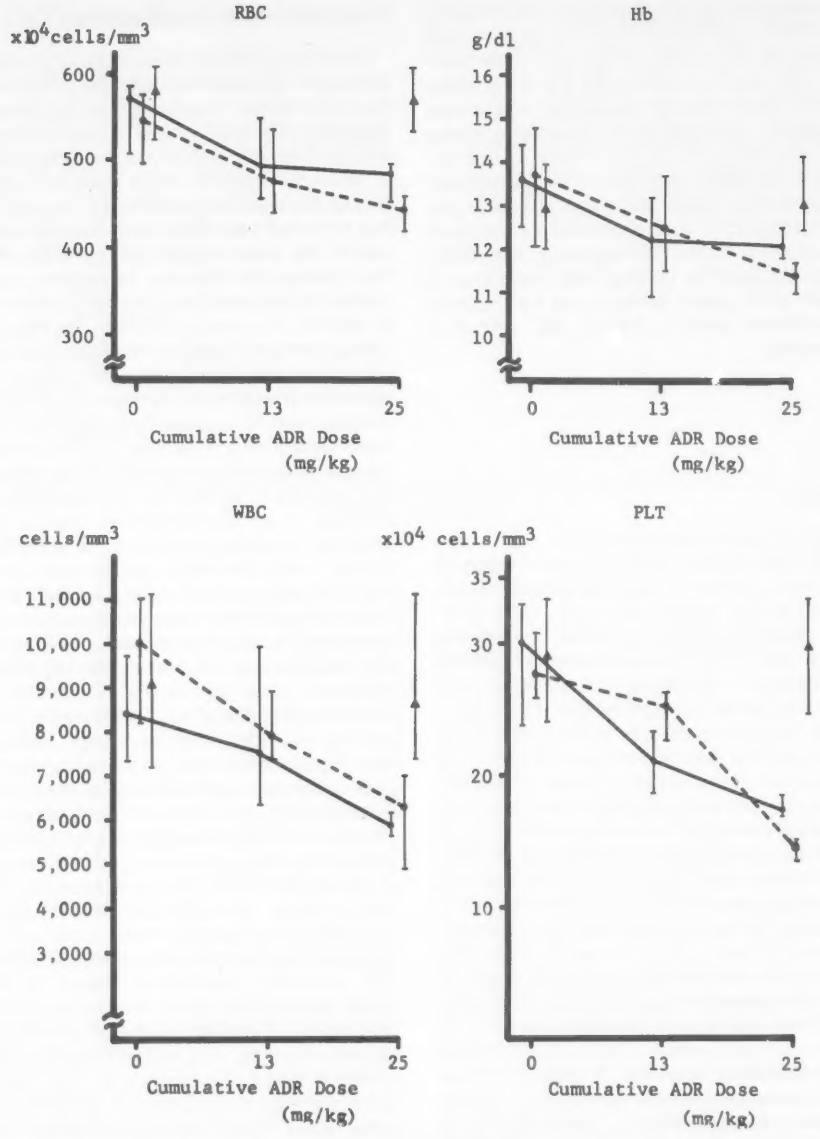


FIGURE 2.—Changes in hematologic findings expressed as mean actual values. Vertical bars = ranges of actual hematologic data on each group. ●—● = group A: doxorubicin, 1.0 mg(0.2 ml)/kg/day iv; ○—○ = group B: doxorubicin, 1.0 mg (0.2 ml) + CoQ_{10}, 2.5 mg(0.25 ml)/kg/day iv; ‡ = groups C and D (control): CoQ_{10}, 2.5 mg(0.25 ml)/kg/day iv and solvent of CoQ_{10}, 0.5 ml/day iv.

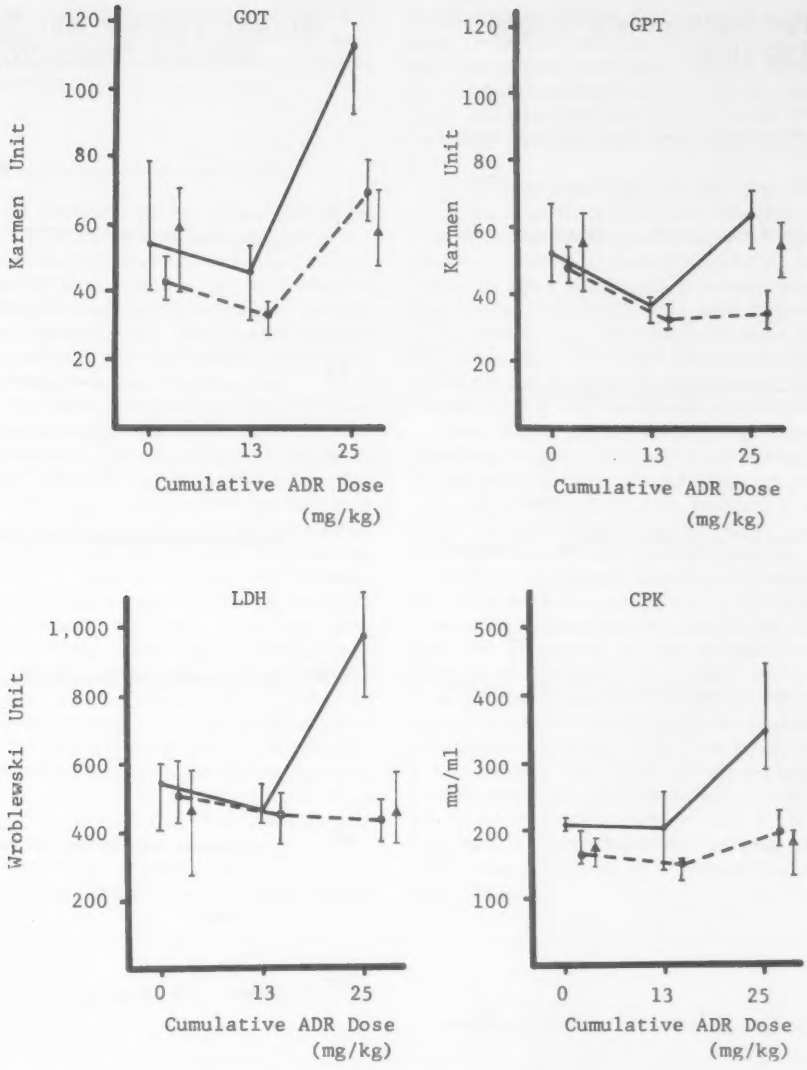


FIGURE 3.—Changes in serum enzyme levels expressed as mean actual values. Vertical bars = ranges of actual chemistry data. ●—● = group A: doxorubicin, 1.0 mg(0.2 ml)/kg/day iv; ○—○ = group B: doxorubicin, 1.0 mg (0.2 ml) + CoQ_{10}, 2.5 mg(0.25 ml)/kg/day iv; ‡ = groups C and D (control): CoQ_{10}, 2.5 mg(0.25 ml)/kg/day iv and solvent of CoQ_{10}, 0.5 ml/day iv.

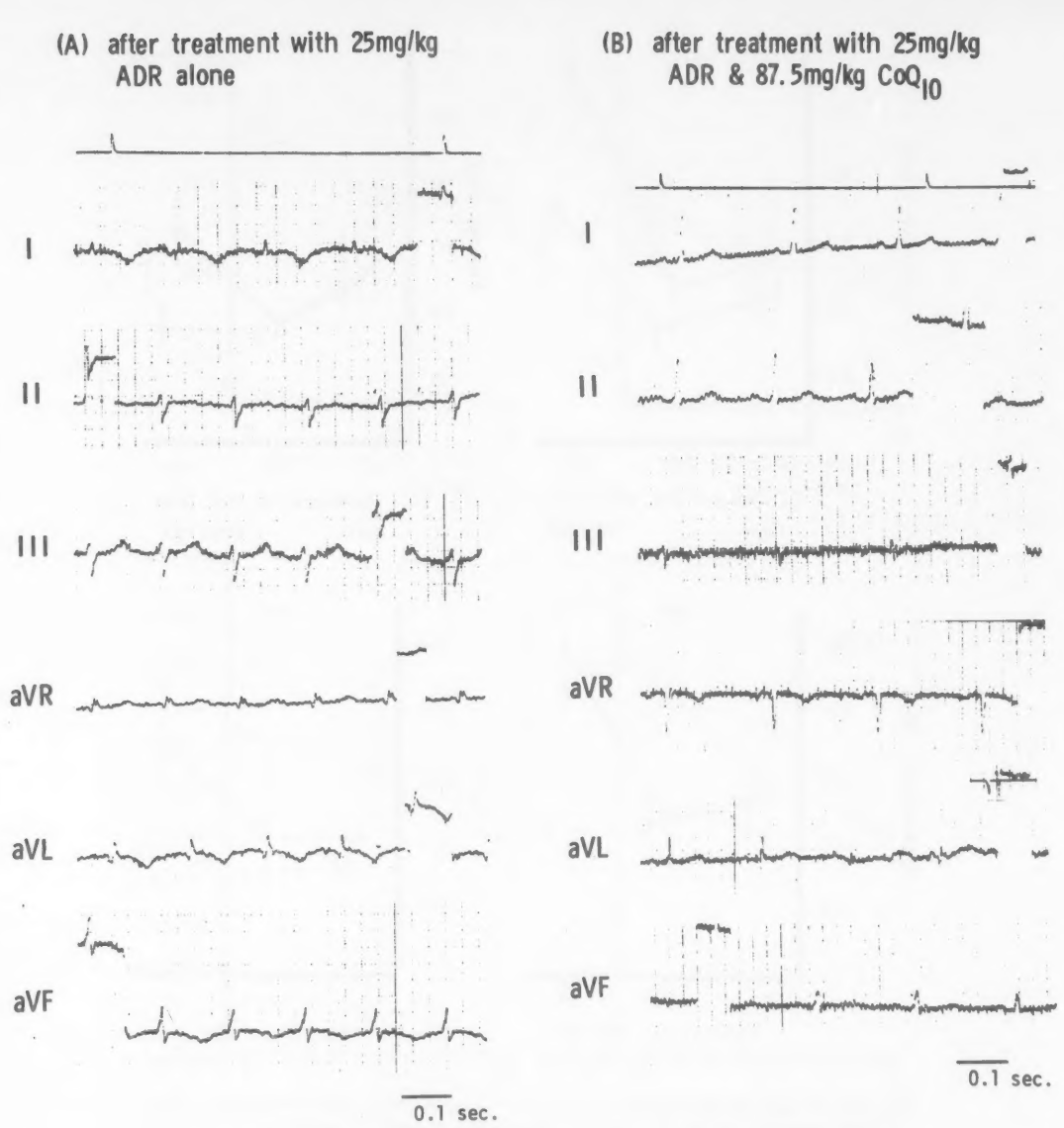


FIGURE 4.—Typical patterns of ECG tracings in doxorubicin-treated rabbits with or without CoQ_{10}. (A) ECG patterns show a decrease in QRS voltage (I, II, III, aVR, and aVL), a flattening of the T wave (II), and inverted T wave (I, aVR, aVL, and aVF) in a rabbit treated with 25 mg/kg of doxorubicin. (B) In contrast, ECG patterns show few changes except for a slight decrease of T-wave amplitude in rabbits treated concomitantly with doxorubicin and CoQ_{10}. The method of ECG tracing is described in the Materials and Methods section.

concomitant administrations of CoQ_{10} may prevent histologic changes in myocardium induced by doxorubicin. A comparative summarization of these ultrastructural findings is shown in table 1.

DISCUSSION

Drug-induced cardiomyopathy, characterized by alterations in the ECG pattern and myocardial degeneration associated with congestive heart failure and other side effects including severe bone marrow suppression, alopecia, and oral ulcerations were observed during chemotherapy for cancer patients receiving the anthracycline antibiotics doxorubicin and daunorubicin.

In man, it has been reported that the ECG abnormalities consist mainly of sinus tachycardia, ventricular extrasystoles, changes in the ST segment, and changes of T wave (9,21). Similar ECG changes were observed in most of the rabbits treated with doxorubicin alone (fig 4A). Except for premature ventricular contractions, arrhythmia was not observed in group A rabbits receiving this chemotherapy. These findings suggest that doxorubicin may injure ventricular myocytes other than conduction systems. This suggestion is further supported by the significant elevation in serum CPK and LDH in the rabbits treated with doxorubicin alone. On the other hand, these ECG and enzyme changes were not found in rabbits given doxorubicin and CoQ_{10} concomitantly (figs 3 and 4B), suggesting that CoQ_{10} is a useful agent for prevention or at least inhibition of cardiomyopathy induced by successive administrations of doxorubicin.

The present histologic studies demonstrated that doxorubicin-induced cardiac injuries were mainly localized in myocytes, as suggested by clinical findings. In rabbits treated with doxorubicin alone, the most prominent histologic changes were observed in the mitochondria in myocytes, and few morphologic changes were seen in the nuclei. These mitochondrial changes were prevented by CoQ_{10} (table 1).

It has been demonstrated (22) that the presence of CoQ_{10} in succinoxidase and NADH-oxidase systems is indispensable for these sites in the essential electron transfer process of respiration and coupled oxidative phosphorylation in mitochondria. It has been shown (12,13,23) that both doxorubicin and CoQ_{10} have a quinone stem in their biochemical structure (fig 1) and that doxorubicin possibly inhibits the action of CoQ_{10} enzymes such as succinoxidase and NADH-oxidase in the mitochondria.

These ultrastructural findings and theories, taken together, suggest that inhibition of enzyme activities in both oxidative phosphorylation and the electron transport system in mitochondria in myocardium plays an important role in the pathogenesis of doxorubicin-induced cardiomyopathy.

Another significant histologic alteration in rabbits treated with doxorubicin alone was that numerous vacuolizations occurred in the capillary endothelial cytoplasm in the myocardium. Similar changes in capillaries were observed by Chalcroft et al (24) and Olson et al (25), suggesting that these alterations in the capillaries in the myocardium may also play a role in the cardiotoxicity of doxorubicin.

In summary, the present study showed that doxorubicin-induced cardiomyopathy, as evidenced by elevation of serum enzyme values, ECG abnormalities, and ultrastructural changes in rabbit myocardium, was prevented by a concomitant administration of CoQ_{10}.

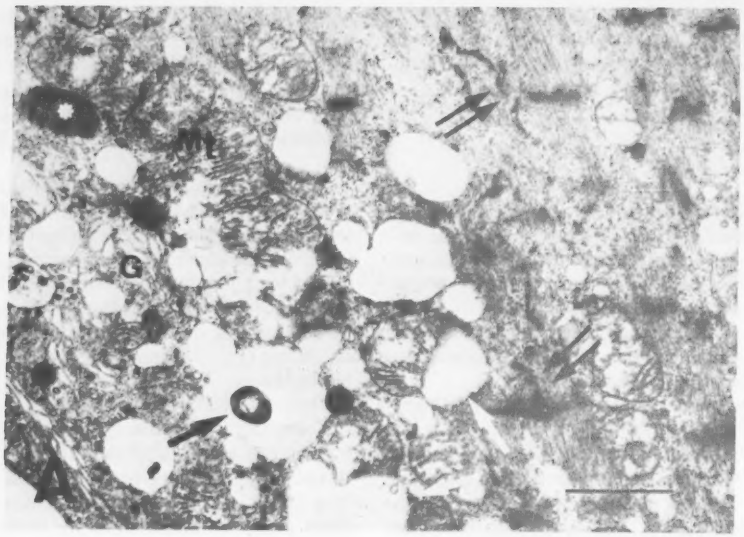
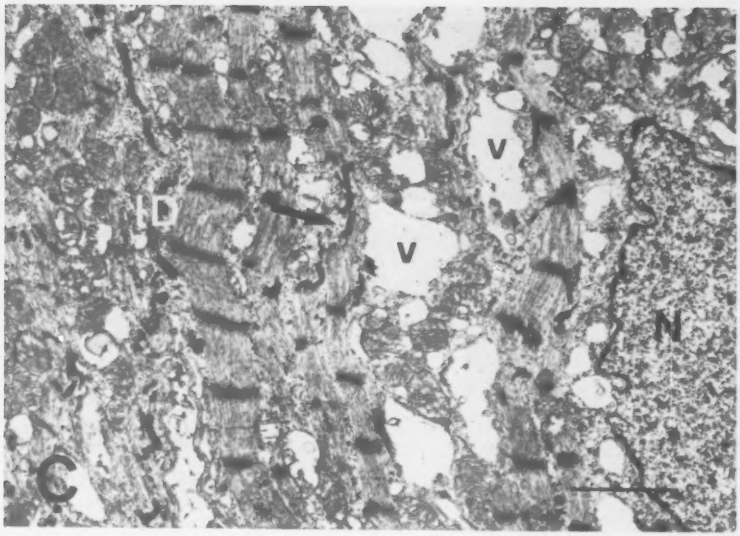


FIGURE 5 (A through K)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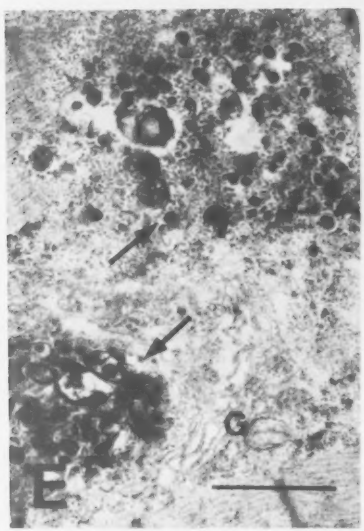
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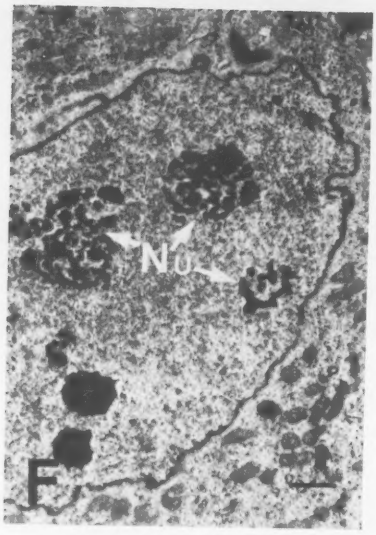
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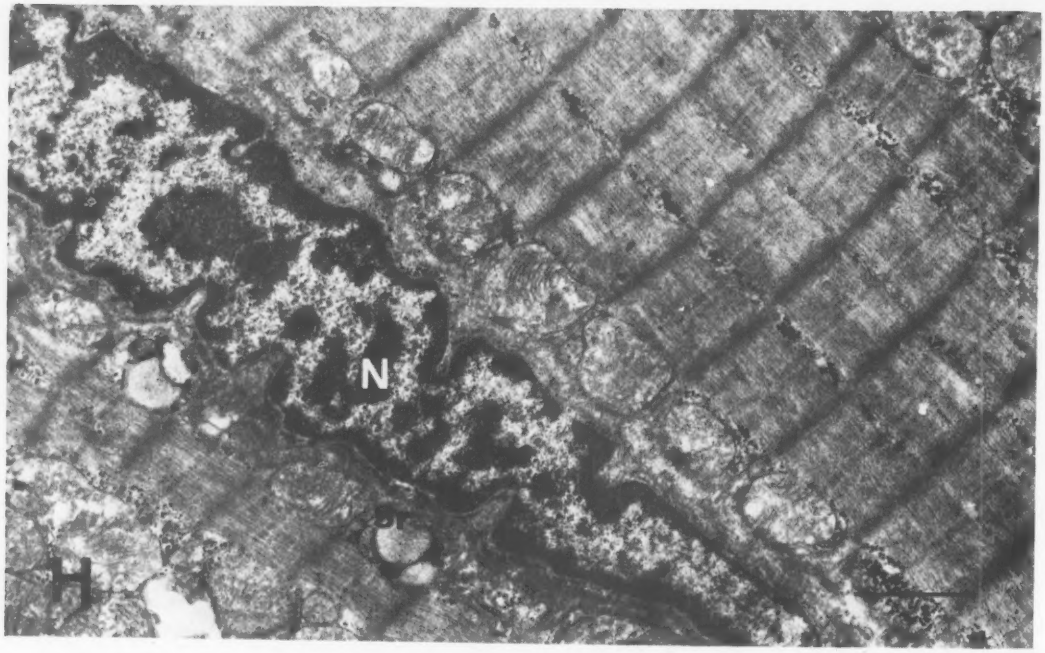
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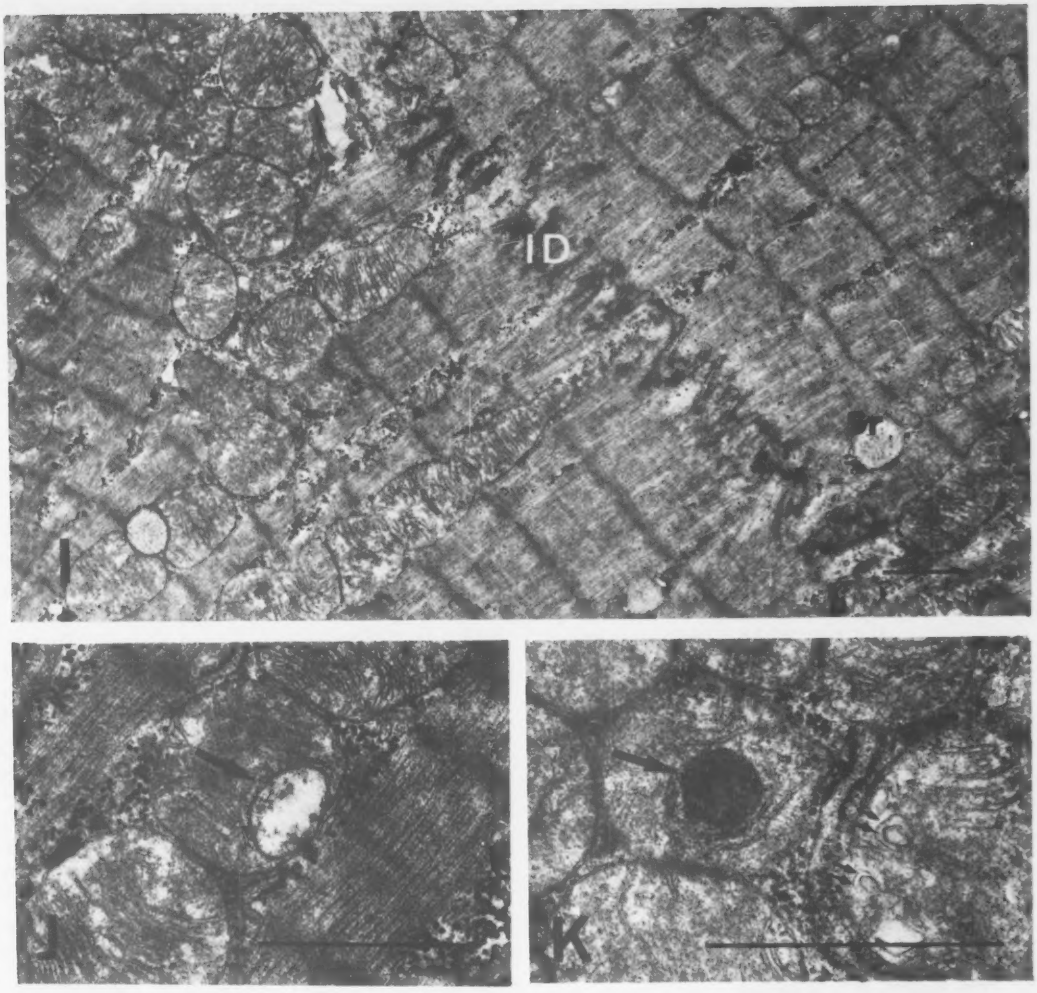

FIGURE 5.—(I) Ultrastructural findings in rabbits treated with 25 mg/kg of doxorubicin alone (group A): **A.** Mitochondrial cristae are lost or disarranged. White arrow indicates the ballooning of outer membrane of mitochondria. Vacuoles and myelin figures (black arrow) appear adjacent to mitochondria, and some mitochondria consist of electron-dense matrix (white asterisk). Double (black) arrows indicate disarrangement of Z-bands; **B.** Severely degenerated mitochondria containing many inclusion bodies were observed; **C.** Electron microscopic picture shows numerous vacuoles in the sarcoplasm and disarrangement of Z-bands (arrow); **D.** Fine filamentous structures close to the myofibrils suggest degenerative change. Ballooning of mitochondrial outer membrane (arrow) is shown; **E.** Extensive deposition of both electron-dense and laminated bodies (arrows) in the sarcoplasm is apparent; **F.** Electron micrography of nucleoli reveals nucleolar segregation (arrows); **G.** Numerous vacuoles in the cytoplasm of capillary endothelium. (II) Ultrastructural findings in rabbits treated with 25 mg/kg of doxorubicin and 87.5 mg/kg of CoQ_{10} (group B): **H** and **I.** Electron micrographs of left ventricular muscle in rabbits simultaneously treated with doxorubicin and CoQ_{10} reveal few abnormalities; **J.** Concentric lamella (arrow) in mitochondria is also detected in rabbit myocardium; **K.** Electron-dense inclusion body in mitochondria is indicated by large arrow. Tiny concentric laminated bodies of mitochondrial cristae (small arrows) are observed. Mt = mitochondria; G = Golgi complex; ID = intercalated disc; V = vacuole; N = nucleus; f = filamentous structure; Mf = myofibril; Nu = nucleolus; Sr = sarcoplasmic reticulum. Scale = 1 μm.

TABLE 1.—Comparison of ultrastructural findings in rabbit myocardium induced by doxorubicin alone and with CoQ_{10}

Ultrastructural findings	Group*,†			
	A	D	C	D
Sarcoplasmic changes				
Decreased glycogen particles	+ +	+	–	–
Vacuolizations in sarcoplasm	+ + +	+	–	–
Z-band abnormalities				
Widening of Z-band	+ +	–	–	–
Disorganization in Z-band arrangement	+ +	–	–	–
Degenerative changes in mitochondria				
Swelling and/or disruption of outer membrane	+ +	–	–	–
Extensive loss and/or disorganization of cristae	+ + +	+	–	–
Formation of numerous concentric lamellae	+ +	+	–	–
Changing into electron-dense matrix	+ +	+	–	–
Partial dissolution of myofibrils	+ +	–	–	–
Extensive deposition of both electron-dense and membranous laminated bodies	+ +	–	–	–
Changes in nuclei				
Clumping of chromatin	+	–	–	–
Segregation of nucleoli	+	+	–	–
Changes in capillary endothelium				
Ballooning and vacuolization in the cytoplasm of endothelial cells	+ +	–	–	–

*Group A: doxorubicin, 1.0 mg/kg/day iv; group B: doxorubicin, 1.0 + CoQ_{10}, 2.5 mg/kg/day iv; group C: CoQ_{10}, 2.5 mg/kg/day iv; and group D: solvent of CoQ_{10}, 0.5 ml/day iv.
†1+ = limited; 2+ = intermediate between 1+ and 3+; 3+ = diffused.

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Thymidine 5′-*O*-pivaloate: Evidence for Prodrug Action in the Rat and Rhesus Monkey[1,2]

A. Rosowsky,* J. E. Wright, G. Steele, and D. W. Kufe[3,4]

SUMMARY

Thymidine (TdR) 5′-*O*-pivaloate was administered to rats and a rhesus monkey to assess its prodrug characteristics. Within 15 minutes after a 2000-mg/kg oral or sc bolus in rats, free TdR was detected in the serum by liquid chromatography. At 1 hour the TdR level reached a plateau of approximately 0.6 mM with oral administration and 0.1 mM with sc treatment. In the monkey experiment, radioactivity was detected in the serum 15 minutes after oral administration of 200 mg/kg of [^{3}H]TdR 5′-*O*-pivaloate. Thin-layer chromatographic analysis showed that this consisted of unchanged ester as well as free TdR and thymine. The concentration of intact ester reached a peak value of 5.4 μM after 40 minutes, before declining to a steady level of 0.9–1.0 μM over the next 6 hours. Serum [^{3}H]TdR and [^{3}H]thymine followed a similar course, except that both remained in the range of 1.0–1.2 μM for as long as 24 hours. Total recovery of radioactivity in the urine over the first 6 hours was 2.5% of the administered dose, with all compounds present in equal proportions. The results of these studies are consistent with absorption of intact pivaloate ester from the stomach, followed by gradual cleavage to TdR and catabolism of TdR to thymine and subsequent products. With a single oral dose of ester, it was possible to achieve, and maintain for up to 24 hours, a level (1 μM) of free TdR in the serum which has previously been found to protect normal tissues during high-dose methotrexate therapy.

[Cancer Treat Rep 65:93–99, 1981]

In recent years, there has been a rising interest in thymidine (TdR) as a selective rescue agent during methotrexate therapy (1–7) and, at high doses, as a potential antitumor agent in its own right (8–15). The biochemical rationale underlying these investigations has been summarized in a comprehensive review (16).

One of the practical disadvantages of TdR as a clinical agent is its very rapid rate of elimination, which necessitates the use of mechanical infusion pumps to maintain therapeutic levels of drug in the tissues for sufficient periods of time. As with other rapidly cleared drugs, a reasonable alternative to achieving sustained high levels is to give TdR in the form of a prodrug derivative. Assuming that chemical and/or enzymatic cleavage of the prodrug occurs at an appropriate rate, it should be possible to simulate continuous-infusion conditions without the attendant risk and inconvenience of mechanical pumps.

A preliminary study in this laboratory has shown that the serum level of free TdR is higher, and remains elevated for a longer time, in mice receiving a single oral or sc dose of TdR 5′-*O*-pivaloate than

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in mice injected with an equimolar amount of TdR itself (17). Superior properties were noted for the ester both in terms of increased uptake of the nucleoside into bone marrow DNA and in terms of prolongation of survival of mice treated with high-dose methotrexate. Because of the well-known high esterase levels in rodents, compared with those in primates (17,18), it was not possible to properly assess the clinical relevance of these findings without first investigating the behavior of the ester in a more appropriate animal model. In the present paper we report experiments on the in vivo disposition of TdR 5′-*O*-pivaloate in the rhesus monkey, whose serum esterase activity is much closer to that of man than that of the mouse (18). Free TdR levels in the serum of rats treated orally or sc with TdR 5′-*O*-pivaloate are also reported and compared with those produced via continuous mechanical infusion of TdR itself (19).

MATERIALS AND METHODS

Pharmacokinetics of TdR 5′-*O*-pivaloate

In Rats

Normal Wistar-Furth rats (weight, 175–200 g) were obtained from Microbiological Associates, Bethesda, MD. The animals were housed two per cage and fed standard laboratory chow. TdR 5′-*O*-pivaloate was prepared as previously described (17) and was administered orally or sc as a 2000-mg/kg bolus in 1:1:8 Emulfor-ethanol-water suspension. Two animals were given the drug orally via a plastic tube, and one was injected sc. At measured time intervals (15, 30, 60, and 90 minutes), blood was drawn into heparinized tubes by ocular puncture, and the rbcs were removed by centrifugation. After addition of 5-bromo-2′-deoxyuridine to a final concentration of 0.02 mM, proteins were precipitated at 4°C with 5% perchloric acid, the pH of the supernatant was adjusted to 7.0 with 4 N potassium hydroxide, and the potassium perchlorate precipitate which crystallized on overnight storage at 4°C was removed to yield a clear solution. The concentration of free TdR was determined by high-pressure liquid chromatographic analysis (17). In a parallel experiment, rats were given TdR by continuous infusion through the jugular vein at a dose of 200 mg/kg/hour (19), and TdR levels were assayed by the same method.

In Rhesus Monkeys

A 5-year-old male rhesus monkey (*Macaca mulatta*) weighing 14 kg was anesthetized by im injection of ketamine (200 mg), and a catheter was inserted surgically into the left axillary artery. The catheter was connected to a three-way valve for drawing blood, maintaining fluids (5% dextrose/saline, 37 ml/hour for 9 hours), or injecting additional ketamine as needed (about 50 mg every 30 minutes). A pediatric bladder catheter was also emplaced to collect urine. [^{3}H]TdR 5′-*O*-pivaloate (2.7 g; specific activity, 0.21 millicurie/mmol) synthesized as previously reported (17), was suspended in 30 ml of sterile water and was given by gastric instillation for 2 minutes. Samples of blood were collected at periodic intervals and rbcs were removed by centrifugation (3000 × *g*). Urine specimens were collected for 2-hour periods. The following analyses were then performed:

(a) Total radioactivity.—Aliquots (100 μl) of serum and urine were added to 5 ml of Aquasol (New England Nuclear Corp, Boston, MA) and the ^{3}H content was determined by scintillation counting (Beckman model LS-335). A linear calibration curve was obtained by adding known amounts of [^{3}H]TdR 5′-*O*-pivaloate to 100 μl of zero-time serum and counting the sample. This allowed the equivalent ^{3}H concentration to be read directly from the calibration curve without the need for a correction for counting efficiency.

(b) Exchange with water.—Portions of selected serum samples were freeze-dried in an apparatus equipped with a dry ice/acetone trap for water collection, and the radioactivity in 100–200 μl of collected water was determined by scintillation counting in 5 ml of Aquasol. Results were adjusted to take into account that water comprises 94% of the serum.

(c) Relative proportions of unchanged ester, TdR, and thymine.—A 400-μl aliquot of each serum sample was diluted with absolute MeOH (600 μl) and kept overnight at 0°C–5°C. The protein precipitate was removed (3000 × *g* for 12 minutes), and the pellet was washed with additional MeOH (400 μl). After a second centrifugation, the combined aqueous alcohol supernatants were transferred to small glass tubes (7.5 × 75 mm) and evaporated under a gentle stream of nitrogen; small volumes of MeOH were used to wash down the sides of the tubes and to ensure that all soluble radioactivity was concentrated in the bottom. The residue from each tube was extracted into MeOH (3 × 20 μl) and was transferred onto a silica gel thin-layer chromatography (TLC) sheet (Eastman 13181, with indicator) along with 5 μl of carrier solution containing 250 μg of nonradioactive TdR 5′-*O*-pivaloate, TdR, and thymine, respectively. After development with 19:1 $CHCl_3$-MeOH, the spots were visualized under an ultraviolet lamp (254 nm), cut out with scissors, and placed with the gel side facing up into 5 ml of Aqua-

TABLE 1.—Comparison of serum TdR levels in rats following a bolus of TdR 5′-*O*-pivaloate or a continuous infusion of TdR

Time (mins)	Concentration (mM)*				
	TdR 5′-*O*-pivaloate (2000 mg/kg)			TdR infusion (200 mg/kg/hr)†	
	Orally		Sc		
15	0.34	0.24	0.14	0.14	0.054
30	0.59	0.39	0.11	0.18	0.067
60		0.57	0.11	0.22	0.077
90		0.57			

*Each column contains the data for a single animal.
†Infusions were performed via the jugular vein as described in ref 19.

sol for counting. As in analysis *a*, a control experiment was carried out to allow the serum level of each compound to be determined directly from a linear plot of counts per minute versus concentration of the appropriate standard.

(d) Radioactivity in the MeOH-insoluble fraction.—The pellet remaining after the second centrifugation in analysis *c* was dispersed in 1 ml of Protosol (New England Nuclear Corp) with the aid of an ultrasonication bath (55°C, 5 hours). Following the addition of three drops of glacial acetic acid, 4 ml of Aquasol was added, and the sample was counted, with an appropriate correction for the difference in counting efficiency between 4:1 Aquasol-Protosol and Aquasol alone.

RESULTS AND DISCUSSION

As shown in table 1, the serum of rats treated with a single 2000-mg/kg oral dose of TdR 5′-*O*-pivaloate already contains free TdR after only 15 minutes. The concentration of free TdR increases to a plateau of approximately 0.6 mM by 1 hour. Similar rapid appearance of free TdR was observed in the serum when the ester was given by sc injection, but the levels achieved via this route were lower than those achieved by oral administration (about 0.1 mM compared to 0.2–0.6 mM). In a parallel experiment, animals receiving TdR itself (200 mg/kg/hour) by jugular vein infusion had levels of 0.05–0.3 mM. In other work published separately (19), we have demonstrated that 0.6-mM levels of serum TdR are produced in rats by infusion of 400–600 mg/kg/hour. Thus, it was clearly possible to achieve serum TdR levels comparable to those obtained via mechanical infusion by giving the nucleoside as a prodrug derivative.

From the data obtained earlier (17), it was calculated that the serum TdR in mice receiving 800 mg/kg of ester orally was about 1 mM at 1 hour and 0.3 mM at 4 hours. However, since the drug in the mouse experiments was given in 10% Tween 80 rather than in 1:1:8 Emulfor-ethanol-water, it was not possible to draw conclusions about the relative ease of oral absorption in the two species. In both the rat and the mouse, high doses of TdR 5′-*O*-pivaloate were found to cause local tissue toxicity which was manifested as a severe ulceration of the skin or gastric mucosa depending on the route of administration. Since pivalic acid itself has been observed to produce similar and even more pronounced ulceration when given sc or orally to mice and rats (W. D. Ensminger, E. M. Egan, and A. Rosowsky, unpublished results), the irritant effect of the pivaloate ester in rodents may be ascribed to the sudden release of a large amount of pivalic acid because of the high esterase activity which is characteristic of these animals. In other mammalian species with lower esterase activity, this would presumably not be a problem since the formation of pivalic acid would be slow enough to avoid the accumulation of toxic concentrations in the tissues. With this in mind, we chose a rhesus monkey as the host animal in which to further evaluate the prodrug characteristics of TdR 5′-*O*-pivaloate.

In general agreement with preliminary observations made previously in mice (17) and in the present study in rats, there was rapid appearance of radioactivity in the serum of the rhesus monkey following gastric instillation of [^{3}H]TdR 5′-*O*-pivaloate (200 mg/kg) in water. Total serum radioactivity increased exponentially over the first 5 hours (fig 1), continued to climb more slowly for about 24 hours, and then began to decline with some radioactivity still being detectable even after 10 days (data not shown). As anticipated on the basis of the known high rate of catabolism of TdR (20), approximately 50% of the counts in the serum after the first 30 minutes consisted of tritiated water, and by 24 hours this had risen to > 90% (table 2). A smaller

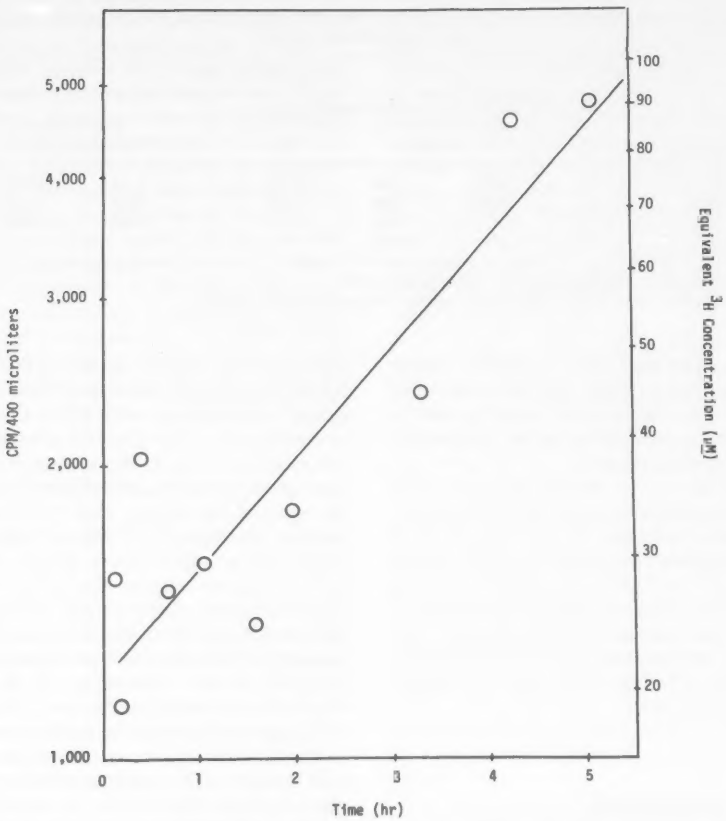

FIGURE 1.—Total serum radioactivity following oral administration of TdR 5′-*O*-pivaloate (200 mg/kg) to a rhesus monkey.

TABLE 2.—Distribution of radioactivity in rhesus monkey serum following oral dose of [^{3}H]TdR 5′-*O*-pivaloate

	Cpm/400 μl*		
Time (hrs)	Total ^{3}H	[^{3}H]H_2O†	MeOH-insoluble ^{3}H†
0.2	1140	—	—
0.4	2020	960 (48)	250 (12)
1.0	1590	—	280 (18)
2.0	1810	—	—
4.0	4660	3110 (67)	370 (8)
24	26,400	24,400 (92)	1850 (7)

*SD = 10%.
†Nos. in parentheses = % of total.

TABLE 3.—Distribution of radioactivity in rhesus monkey urine following oral dose of [^{3}H]TdR 5′-*O*-pivaloate

Collection period (hrs)	Volume collected (ml)	Total ^{3}H		Concentration (μM)*,†		
		Cpm/100 μl*	Cumulative % of dose	TdR 5′-*O*-pivaloate	TdR	Thymine
0–2	20	25,500	0.45	55 (16)	135 (39)	158 (45)
2–4	20	15,500	0.74	25 (21)	40 (33)	56 (46)
4–6	20	29,400	1.27	30 (16)	54 (28)	107 (56)

*SD = 10%.
†Nos. in parentheses = % of total TdR 5′-*O*-pivaloate, TdR, and thymine.

radioactive component was also present in the MeOH-insoluble fraction. This presumably represents tritium incorporated into protein by exchange, rather than protein-bound drug. Control experiments showed that any labeled TdR ester, TdR, or thymine would have been removed effectively from the protein during washing of the protein pellet with MeOH (*see* Materials and Methods section).

Analysis of the deproteinized serum by TLC revealed the presence of intact ester at every time point, along with some TdR and thymine (fig 2). The ester concentration reached a peak value of 5.4 μM after 40 minutes, thereafter declining to an approximately constant level of 0.9–1.0 μM over 6 hours. The early appearance of ester in the serum attests to the ability of this compound to be absorbed intact via the oral route. Sustained mode of

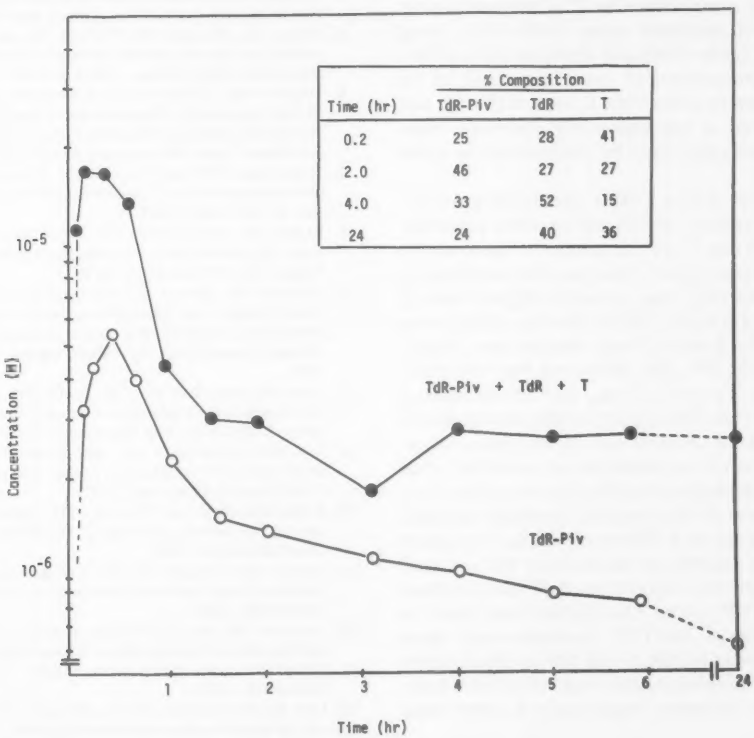

FIGURE 2.—Serum levels of TdR 5′-*O*-pivaloate, TdR, and thymine in a rhesus monkey following oral administration of the pivaloate ester (200 mg/kg).

action was indicated also by the fact that the ester could be detected even at 24 hours and by the fact that the proportions of intact ester, TdR, and thymine in the serum did not change markedly during the experiment. This is what would be expected if TdR were being released continuously from the ester. Incubation of the ester with TdR phosphorylase showed that this compound does not undergo cleavage of the glycoside bond to give thymine (G. P. Beardsley, unpublished data). Therefore, it is very likely that any thymine in the serum originates from TdR rather than directly from the pivaloate ester. However, the present results do not allow a distinction between hydrolysis of the pivaloate by serum esterases and hydrolysis in the gastrointestinal lumen, in gastrointestinal cells, or in the liver.

Urinary excretion of radioactivity (table 3) was quite low over the first 6 hours, averaging approximately 0.4%/hour. On a cumulative basis, this amounted to $< 2.5\%$ of the administered dose, which was consistent with previous findings in human subjects receiving TdR by infusion (5). Analysis of the urine composition by TLC showed that it contained intact pivaloate ester (16%–21%), along with free TdR (28%–39%) and thymine (45%–66%). The relative proportion of ester appeared to be somewhat lower in urine than in serum (fig 2), and thymine showed a corresponding increase, suggesting that the ester may be reabsorbed to some extent.

The peak level of free TdR in the monkey following oral administration of 200 mg/kg of the pivaloate ester was 0.006 mM (approximately the same as the peak level of intact ester), whereas the corresponding value in rats receiving a tenfold higher dose of 2000 mg/kg was 0.6 mM. When the two doses were compared on the basis of body surface area rather than body weight (21), the difference was only fivefold (12 versus 2.4 g/m^2). Thus, the 100-fold-lower peak level of serum TdR in the monkey as compared to the rat could not be explained on the basis of dose alone. Several other explanations are possible, none of which are mutually exclusive: (*a*) absorption may have been slower in the monkey, perhaps because the drug was given in a different vehicle; (*b*) higher serum esterase activity in the rat may have caused nearly instantaneous conversion of all the absorbed drug to free TdR; (*c*) at the higher dose used in rats, the pathways for TdR clearance may have become saturated (14,19); or (*d*) tissue distribution of the lipophilic pivaloate ester may have been more extensive in the monkey, resulting in a lower drug level in serum.

It is also of interest that the serum level of free TdR in the monkey was still approximately 1.2 μM even at 24 hours (fig 2). TdR levels in the zero-time serum of the monkey were found to be below the level of sensitivity of the high-pressure liquid chromatography assay, which is estimated to be 0.3 μM. In man, the endogenous TdR level, as measured by radioimmunoassay, is reported to be 0.16 μM (11). Although a serum TdR level in the range of 1–2 μM would not be sufficient to elicit therapeutic benefit when TdR is used by itself (12), a level of 1.5 μM does prevent bone marrow toxic reactions in patients receiving high-dose methotrexate (5). On this basis we feel that prodrugs of TdR—of which 5′-*O*-pivaloate ester is but one possible example—have potential applications as orally absorbed protective agents for use during antifolate chemotherapy.

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Disposition of 5-Methyltetrahydrohomofolate and Methotrexate in Rats[1,2,3]

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SUMMARY

The pharmacologic disposition of [*methyl*-^{14}C]5-methyltetrahydrohomofolate (MTHHF) and [^{3}H]methotrexate (MTX) has been studied in rats after iv doses of 25 mg/kg. Thin-layer chromatography and high-pressure liquid chromatography were used to separate the parent compounds from possible products. In rat serum, levels of MTHHF decreased with half-lives of 7 and 55 mins; for MTX, the corresponding values of half-lives were 14 and 54 mins. No metabolites of MTHHF or MTX were found in the samples assayed. At 3, 6, and 24 hrs after administration of MTHHF or MTX, liver, kidney, and small intestine contained concentrations of the drugs greater than those in serum. In rats with cannulated bile ducts, 16% and 60% of the doses of MTHHF and MTX, respectively, were excreted in the bile within 4 hrs. In 24 hrs, 51% of the dose of MTHHF and 41% of the dose of MTX were excreted in the urine of rats without bile cannulas. During the same time, fecal excretion accounted for 21% of the dose of MTHHF and 17% of the dose of MTX. The data indicate that enterohepatic circulation is probably extensive for MTX but less so for MTHHF.

[Cancer Treat Rep 65:101–106, 1981]

dl-5-Methyltetrahydrohomofolate (MTHHF), which has an undefined site of action, has considerable chemotherapeutic activity against L1210 leukemia/FR-8 (1), a variant resistant to folate antagonists (2). In Ehrlich ascites cells, there is a distinct and rapid transport mechanism for MTHHF (3). We have recently completed a study on the disposition of [^{14}C]MTHHF in mice, dogs, and monkeys (4). The compound, which was not metabolized to detectable products, was excreted primarily in the urine and, to a lesser extent, in the feces of these animals. In the present investigation, we have extended these studies on MTHHF to rats, determining its serum and tissue levels and the extent of biliary, urinary, and fecal excretion and comparing its disposition with that of methotrexate (MTX), a widely used folate antagonist, given as an equivalent dose.

MATERIALS AND METHODS

Bio-Solv BBS-3 solubilizer was purchased from Beckman Instruments, Palo Alto, CA, and Liquifluor and Aquasol scintillation solutions were purchased from New England Nuclear Corp, Boston,

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[2]Supported by contract N01-CM-87162 from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3]5-Methyltetrahydrohomofolate, [*methyl*-^{14}C]5-methyltetrahydrohomofolate, and methotrexate were supplied by Dr. J. A. R. Mead, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD. [3′,5′,9(*n*)-^{3}H]Methotrexate was purchased from Amersham/Searle Corp, Arlington Heights, IL.

[4]Kettering-Meyer Laboratory, Southern Research Institute, Birmingham, AL.

[5]We are grateful to Dr. David Farquhar of the M. D. Anderson Hospital and Tumor Institute, Houston, TX, to Dr. David Johns of the National Cancer Institute, Bethesda, MD, for samples of 7-hydroxymethotrexate, and to Dr. Robert Piper of Southern Research Institute for samples of 4-amino-4-deoxy-N^{10}-methylpteroic acid and MTX-diglutamate. We also thank Mr. Thomas C. Herren for radioassays, Mr. Glenn Duncan for work involving high-pressure liquid chromatography, and Mr. William Gardner for technical assistance.

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MA. Type SA thin-layer sheets were obtained from Gelman Instrument Co, Ann Arbor, MI. All radioactive samples were counted in a liquid scintillation spectrometer equipped with an external standard. The radiochemical purity of [^{14}C]MTHHF (0.9 microcurie/mg) was 97%–100%, as determined by thin-layer chromatography and high-pressure liquid chromatography, and that for [^{3}H]MTX (9.4 microcuries/mg) was 95%, as determined by high-pressure liquid chromatography. The major impurity in the MTX preparation comprised about 3% of the total radioactivity.

Animals used in this study were male Sprague-Dawley rats weighing 150–600 g. Prior to dosing, the rats were starved overnight, although water was provided. Iv doses of MTHHF in 0.9% sodium chloride and MTX in 2% sodium bicarbonate were 25 mg/kg (150 mg/m^2). In mice, 50 mg/kg (150 mg/m^2) of MTHHF is a therapeutic dose.[6]

For collection of bile, rats were anesthetized with diethyl ether, and the common bile duct was cannulated. From restrained animals, urine was collected by catheter, and feces was collected on aluminum foil. Blood samples were obtained from the femoral vein. Various organs and tissues of rats were collected separately, blotted on filter paper, and placed immediately on dry ice. After being thawed and weighed, the tissues were homogenized in 4 vol of 0.9% sodium chloride with a Brinkman Polytron equipped with a P-10 generating head (Brinkman Instruments, Westbury, NY). The 0.9% sodium chloride used for homogenization of tissues of rats injected with MTHHF also contained 5% ascorbate, a stabilizing agent.[6] MTHHF and biologic samples from rats given this drug were protected from light and not exposed to heat. For determination of total radioactivity in biologic samples, 15 ml of Liquifluor containing Bio-Solv BBS-3 (14:1, vol/vol) was added to 0.1 ml of sample and counted.

Portions (2–100 μl) of serum, bile, and urine from rats injected with [^{14}C]MTHHF were spotted on type SA sheets. The chromatograms were developed to 15 cm in a solvent of *n*-butanol:acetic acid:water (20:3:7, vol/vol/vol). Unlabeled MTHHF, visualized by ultraviolet light at 250 nm, was used as the reference compound. After drying, the sheets were cut into 0.6-cm strips, starting 0.6 cm behind the origin and ending about 1 cm beyond the solvent front. The strips were placed in counting vials containing 1 ml of H_2O for 30 mins before the addition of 10 ml of Aquasol. Radioactivity migrating with unlabeled MTHHF (R_f 0.49) represented the compound. In vitro, MTHHF was stable for 24 hrs in water or serum.

Serum, bile, and urine samples containing MTX were injected directly into a Waters' high-pressure liquid chromatograph (model ALC/GPC-202) equipped with a Whatman Partisil ODS-2 column, a model 440 absorbance detector set at 254 nm, and a Hewlett-Packard 3380A integrator. The solvent was 85% 0.1 M sodium acetate (pH 3.6) and 15% acetonitrile, and the flow rate was 1 ml/min. Selected biologic samples from rats injected with MTHHF were also assayed by this procedure. With this system, the retention time was 8.5 mins for MTX and 15 mins for MTHHF.

Serum half-lives for MTHHF and MTX were estimated as described previously (4). Since metabolism was minimal, tissue contents of the two drugs were calculated from the total radioactivity present.

RESULTS AND DISCUSSION

Following iv injection of 25 mg/kg, [^{14}C]MTHHF disappeared from the serum of rats in two phases with half-lives of 7 and 55 mins (fig 1). These values are similar to those observed for the mouse, dog, and monkey (4). It is possible that a third phase, as observed for dogs and monkeys (4), could have been detected with a preparation of [^{14}C]MTHHF having higher specific activity. In the parallel study with [^{3}H]MTX, the serum half-lives were found to be 14 and 54 mins (fig 1). In mice, MTX, given either ip or sc, has a half-life of 1 hr (5). The serum levels of MTX were consistently lower than those for MTHHF (fig 1). MTHHF was found in erythrocytes at a concentration equal to that in serum.

As was previously observed for mice, dogs, and monkeys (4), no appreciable amount of metabolites of [^{14}C]MTHHF in serum, bile, or urine samples was detected, either on thin-layer chromatography or on high-pressure liquid chromatography. Furthermore, the ultraviolet spectrum for MTHHF isolated from urine was identical to that for the authentic compound. An impurity in the preparation of [^{3}H]MTX (about 5% of the radioactivity present) was noted in the urine and bile but at amounts < 2% of the radioactivity in the serum and tissues of rats. The impurity is apparently not bound intracellularly but is excreted rapidly in the urine and bile. It was distinguished by both types of chromatography, from authentic 7-hydroxymethotrexate, a known metabolite of MTX (6), and from 4-amino-4-deoxy-N^{10}-methyl pteroic acid, a product of MTX formed by the intestinal flora (7). We concluded that essentially all (> 98%) of the radioac-

[6]Unpublished results, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD.

TABLE 1.—Tissue levels of MTHHF and MTX in rats following iv dose of 25 mg/kg*

Tissue	Tissue levels (μg/g)						
	MTHHF			MTX			
	3 hrs	6 hrs	24 hrs	1 hr	3 hrs	6 hrs	24 hrs
Liver	15.0	19.1	15.6	15.5	3.9	2.4	1.5
Kidney	24.01	28.1	11.3	37.8	196.0	84.3	24.0
Spleen	2.8	2.9	3.8	1.6	1.4	0.6	0.3
Pancreas	7.9	6.6	5.0	1.8	0.8	0.6	0.2
Lung	3.1	3.1	3.7	2.6	0.9	0.7	0.3
Small intestine	120	27.7	9.1	23.0	43.9	275.0	59.7
Large intestine	3.5	4.8	7.5	2.1	0.9	1.3	0.5
Cecum	3.0	3.3	3.8	2.1	0.7	0.9	0.4
Small intestine contents	ND†	ND	ND	1060	1810	2640	3990
Large intestine contents	ND	ND	ND	0.2	< 0.1‡	< 0.1	< 0.1
Cecum contents	ND	ND	ND	< 0.1	1.6	0.8	0.1
Muscle	1.0	1.0	1.0	1.1	0.6	0.2	0.2
Testis	1.5	1.0	2.1	2.1	0.9	0.4	0.3
Brain	0.7	0.8	1.0	0.3	0.3	0.1	0.1
Thymus	ND	ND	ND	1.8	1.2	1.0	0.3
Heart	ND	ND	ND	1.6	0.6	0.3	0.2
Stomach	ND	ND	ND	2.1	0.9	ND	0.3

*No bile was collected from these rats.
†ND = not determined.
‡Lower limit for detection of MTX was 0.1 μg/ml (or g); that for MTHHF was 0.5 μg/g.

tivity present in the tissues of rats injected with either [^{14}C]MTHHF or [^{3}H]MTX would be unchanged drug or polyglutamates which are converted back to the parent compounds prior to, or shortly after, their exit from the cells (8–13). No MTX diglutamate, which inhibits dihydrofolate reductase similarly to MTX (10), was observed in the serum, bile, or urine of the rats by high-pressure liquid chromatography with an authentic standard.

At 3 hrs after administration of [^{14}C]MTHHF, all tissues examined, except the liver, kidney, and small intestine, had concentrations lower than that in serum (table 1). The results are similar to those for mice at 3 hrs and dogs and monkeys at 1 and 6 hrs after injection (4). The concentration in liver at 24 hrs remained at a level equivalent to that at earlier times. The same was true for the lower concentrations in spleen, pancreas, lung, cecum, muscle, testis, and brain. The high concentration in the small intestine dropped markedly between 3 and 24 hrs. To a smaller extent, the concentration in the kidney also dropped. Except for kidney and small intestine, tissue concentrations of MTX were lower than those for MTHHF. MTX in all tissues except small intestine, dropped between 3 and 24 hrs, in contrast to MTHHF. MTX is known to accumulate in the small intestine of mice (14,15).

Biliary excretion of unchanged [^{14}C]MTHHF in 24 hrs accounted for 16% of the dose administered (fig 2). In contrast, 60% of the dose of MTX was excreted in the bile within this time. Biliary excretion of both compounds was essentially complete at 4 hrs after injection (fig 2). Biliary excretion and enterohepatic circulation of MTX in rats have been previously noted (16–18). In a previously reported experiment, 57% of a 20-mg/kg iv dose of MTX was excreted in the bile of a rat in 8 hrs (16). Since the percent of the dose of MTHHF excreted in the bile was equivalent to that found in the feces, any appreciable absorption of MTHHF from the small intestine would have to be balanced by further cycling of the drug into the bile. The extent of biliary excretion of MTHHF by mice given ip doses was not determined, but the percent of the dose excreted in the feces in 24 hrs (4) was essentially the same as that for the rat. Since for dogs the percent of dose excreted in the feces was small, these animals may excrete less MTHHF into the bile. Accordingly, assays of the MTHHF content of bile taken from animals at 1 and 6 hrs after injection revealed considerably less in the bile of the dog than in that of the monkey (4).

Within 6 hrs after MTHHF was injected into rats, 41% of the dose had appeared unchanged in the urine; within 24 hrs, 51% had been excreted by this route. Corresponding values for MTX were 36% and 41% (table 2). Values previously reported for the 24-hr urinary excretion of MTX (administered ip) range between 37% and 50% (16). The amount of MTX excreted in the urine was considerably lower

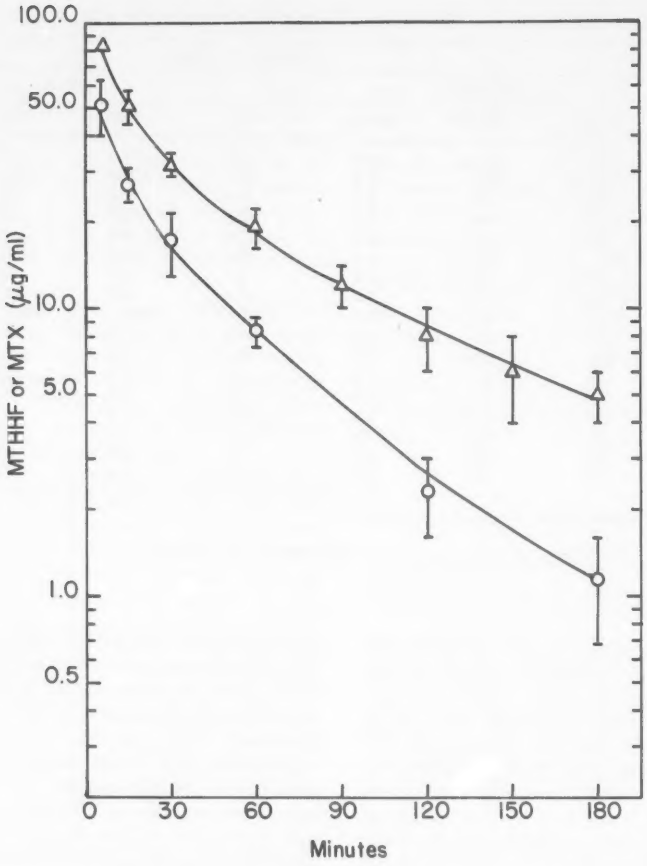

FIGURE 1.—Serum levels of [^{14}C]MTHHF and [^{3}H]MTX in rats following equivalent iv doses of 25 mg/kg. ○ = MTX; △ = MTHHF. 3 rats were used for each drug, and no bile was collected. Bars = SDs.

in rats with biliary cannulas. Fecal excretion of the two drugs in the 24-hr period was not greatly different: 21% of the dose for MTHHF compared to 17% of the dose for MTX. Values previously reported for the 24-hr fecal excretion of MTX (administered ip) are 29% and 41% (16).

The data presented here demonstrate that, although overall excretion into urine and feces is similar for MTHHF and MTX, MTHHF is distributed differently in the bodies of rats. Due to slower and less extensive biliary excretion, concentrations of MTHHF in serum and most tissues of rats remain higher than those of MTX. An exception is the small intestine, in which high concentrations of MTX are found and in which MTX has a major toxic effect. Persistence of MTHHF in most of the tissues of animals could contribute to the therapy for tumors in these organs.

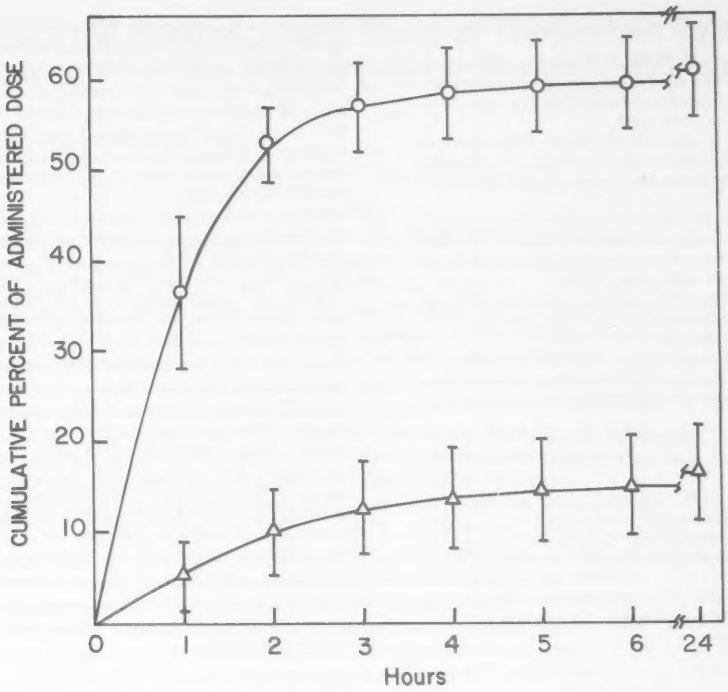

FIGURE 2.—Cumulative biliary excretion of [^{14}C]MTHHF and [^{3}H]MTX by rats following equivalent iv doses of 25 mg/kg. ○ = MTX; △ = MTHHF. 4 rats were used to study biliary excretion of MTHHF, and 5 were used for MTX. Bars = SDs.

TABLE 2.—Urinary and fecal excretions of MTHHF and MTX by rats following iv dose of 25 mg/kg

Collection period (hrs)	% (± SD) recovery of total dose administered			
	MTHHF		MTX	
	Urine	Feces	Urine	Feces
0–3			31.8 ± 6.7 (2)*	
3–6			4.8 ± 1.3 (2)	
6–7			2.8 ± 0.1 (2)	
7–24			1.7 ± 0.8 (2)	
0–6	40.5 ± 6.3 (3)			
6–12	6.2 ± 4.4 (3)			
12–24	1.4 ± 1.1 (3)			
0–24	50.9 ± 5.6 (8)	20.9 ± 0.8 (4)	41.1 ± 4.6 (2)	17.3 ± 10.1 (2)
0–24			14.6 ± 3.6 (5)†	

*Nos. in parentheses = No. of rats used for each determination.
†Bile ducts of these rats were cannulated.

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Biochemical and Pharmacologic Study of Therapeutic Synergism With Cyclophosphamide Plus Methotrexate in Murine L1210 Leukemia[1,2]

Philip Klubes* and Ingeborg Cerna[3]

SUMMARY

The combination of cyclophosphamide (CP) plus methotrexate (MTX) was found to be therapeutically synergistic against the L1210 ascites tumor. After inoculation with 1×10^6 L1210 cells to $(C57BL/6 \times DBA/2)F_1$ mice, ip administration of CP (200 mg/kg on Day 5) plus MTX (15 mg/kg on Days 5, 7, 9, and 11) resulted in a significant increase in mean lifespan, compared to optimal treatment with either CP or MTX alone. The contribution of the single, simultaneous dose of CP plus MTX on Day 5 to therapeutic synergism was examined. The combination of CP plus MTX on Day 5 produced a greater decrease in tumor cell numbers than either drug alone. Measurements of the time course of inhibition and recovery of ^3H-deoxyuridine incorporation into DNA showed that within 48 hours, recovery of DNA synthesis in small intestine and bone marrow was almost complete after either CP, MTX, or CP plus MTX. In contrast, tumor, which had recovered within 48 hours after MTX, still remained > 90% inhibited after either CP or CP plus MTX. Measurements of bone marrow nucleated cellularity after therapy showed that the single, simultaneous dose of CP plus MTX was no more toxic to bone marrow than CP alone, although it was more toxic than MTX alone. No alterations in the distribution of either MTX or alkylating metabolites of CP could be detected in tumor or normal tissues when CP and MTX were administered simultaneously.

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The antimetabolite methotrexate (MTX) and the alkylating agent cyclophosphamide (CP) have been successfully used clinically both as single agents and as components of multiple-drug combinations (1–3). Straus et al (4,5) have shown that the sequence of administration of CP and MTX to mice bearing L1210 leukemia altered the effectiveness of the combination in that longer survivals were noted when CP was administered prior to MTX. In addition, their results indicated that a high priming dose of either CP or MTX lowered the tumor cell population so that subsequent doses of MTX killed the remaining tumor cells more effectively. However, further studies were not done to characterize this effect. This report describes some of the biochemical and pharmacologic effects of MTX and CP when used alone and in combination against mice bearing L1210 ascites tumor.

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MATERIALS AND METHODS

Chemicals

MTX was obtained as a gift from L. H. Kedda, Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD. CP was obtained as a gift from Mead

Johnson and Co, Evansville, IN. ^{3}H-(6)-deoxyuridine (^{3}H-dUrd) (28 curies/mmol) was obtained from the New England Nuclear Corp, Boston, MA.

Animals and Tumor

Male (C57BL/6 × DBA/2)F_1 (hereafter called B6D2F_1) mice, weighing 18–22 g, and male DBA/2 mice, weighing 18–20 g, were obtained from Sprague-Dawley Laboratories, Madison, WI. Animals were caged in an air-conditioned room lighted from 8 AM to 8 PM and had free access to a standard Purina laboratory chow diet and tap water. The L1210 tumor in the ascites form, obtained from Hazelton Laboratories, Falls Church, VA, was maintained in male DBA/2 mice. All experiments were carried out with male B6D2F_1 mice.

Chemotherapeutic Agents

CP was dissolved in 0.9% NaCl just prior to injection. MTX was dissolved in 2% $NaHCO_3$ just prior to injection. All drugs were injected ip at a volume of 0.01 ml/g of mouse body weight.

Effect of Chemotherapy on Survival of Mice Bearing L1210 Ascites Cells

Chemotherapy was initiated on Day 5 following ip inoculation of 1×10^6 L1210 cells. In the survival experiments, groups of ten mice each received either (*a*) 0.9% NaCl (control); (*b*) MTX (7.5, 15, 30, or 60 mg/kg on Days 5, 7, 9, and 11); (*c*) CP (200, 300, or 400 mg/kg on Day 5); or (*d*) CP (200 mg/kg on Day 5) plus MTX (7.5 or 15 mg/kg on Days 5, 7, 9, and 11). Animals were observed daily, and the mean survival time was determined as previously described (6).

Effect of Chemotherapy on Tumor Cell Numbers

Mice were given an ip injection of 1×10^6 L1210 ascites cells on Day 0 and were randomly allocated into either a control group or CP-, MTX-, or CP plus MTX-treated groups. On Day 5 after tumor inoculation, which was during the late logarithmic phase of tumor growth, animals received a single ip injection of either CP (200 or 300 mg/kg), MTX (15 mg/kg), or CP (200 mg/kg) plus MTX (15 mg/kg). Control animals received an equivalent volume of 0.9% NaCl. Forty-eight hours after drug treatment, the animals were killed by cervical dislocation. Laparotomies were performed, and the ascites cells were obtained by flushing the peritoneal contents with 10 ml of ice-cold 0.9% NaCl into a heparinized 50-ml centrifuge tube. The cells were suspended in 20 ml of ice-cold 0.2% NaCl for 30 seconds to hemolyze any erythrocytes present. The suspension was then adjusted to isotonicity by the addition of 4 ml of ice-cold 4.5% NaCl and centrifuged for 5 minutes at 1000 × *g*. The tumor cells were resuspended in ice-cold 0.9% NaCl. The number of ip tumor cells from each of four mice per treatment group was determined using a model 2F Coulter electronic particle counter. Results are expressed as the mean of four determinations ± SE per treatment group.

Assay of MTX and the Incorporation of ^{3}H-dUrd Into DNA

Mice bearing a Day-5 ascites tumor were given ip injections of either CP (200 mg/kg), MTX (15 mg/kg), or CP (200 mg/kg) plus MTX (15 mg/kg). At specific time intervals, the animals received 200 microcuries/240 mg/kg of ^{3}H-dUrd sc, and 20 minutes later, when the incorporation of radioactivity into DNA was still linear with respect to time, the animals were killed by cervical dislocation. Tumor cells from two mice per group were harvested in ice-cold 0.1 M phosphate-buffered saline (PBS) (pH 6.0), pooled, centrifuged, and resuspended in 5 ml of cold PBS. The 5-ml suspension of tumor cells was divided in half for analysis of DNA specific activity and MTX content. A segment (1–1.5 inches) of upper small intestine was obtained from two mice per group, washed with ice-cold 0.1 M PBS (pH 6.0), pooled, and homogenized in 5 ml of ice-cold PBS. The homogenate was then divided in half for analysis of DNA specific activity and MTX content. Bone marrow was flushed from two hind leg femora with cold 0.1 M PBS (pH 6.0), and suspensions from a total of four femora from two mice were pooled in a final volume of 5 ml and were divided in half for analysis of DNA specific activity and MTX content. To determine DNA specific activity, the suspensions of either tumor or bone marrow cells or homogenate of upper small intestine were mixed with an equal volume of cold 20% trichloroacetic acid. The DNA present in the trichloroacetic acid-insoluble precipitate was extracted as previously described (7). The DNA content of a 0.5-ml aliquot of the extract was determined by the diphenylamine method (8), using calf thymus DNA as a standard, and the radioactivity was counted in Scintiverse with a Beckman DPM-100 liquid scintillation spectrometer. Counting efficiency was determined by the external standard method, and the results were expressed as disintegrations per minute (dpm) per microgram of DNA.

For comparison, the incorporation of ^{3}H-dUrd into DNA of saline-treated tumor-bearing mice was measured at Time 0 on Day 5 after tumor inoculation. The incorporation of ^{3}H-dUrd into tumor, upper small intestine, and bone marrow was 224, 75, and 57 dpm/μg of DNA, respectively. Each point represents the mean ± SE of four determinations, with two mice per determination. For determination of MTX content, the sample of tumor cells, small intestine, or bone marrow cells in 2.5 ml of cold PBS was placed in a boiling water bath for 10 minutes. The sample was chilled on ice and centrifuged; the supernatant was frozen and stored for subsequent analysis. MTX was determined with an MTX radioassay kit (New England Enzyme Center, Boston, MA) which uses ^{125}I-MTX and dihydrofolate reductase. This assay system is based on the competitive binding of labeled and unlabeled MTX to *Lactobacillus casei* dihydrofolate reductase, according to the method of Myers et al (9).

Assay of CP Alkylating Activity

Groups of mice bearing a Day-5 ascites tumor received a single ip injection of either CP (200 mg/kg) or CP (200 mg/kg) plus MTX (15 mg/kg), and four mice per treatment group were killed by decapitation at 10, 20, 60, 120, and 180 minutes after drug administration. Alkylating activity in either tumor cells or upper small intestine was determined with 4-(*p*-nitrobenzyl)pyridine as previously described (10,11).

Effect of Chemotherapy on Bone Marrow Nucleated Cellularity

Normal non-tumor-bearing mice, in groups of four, were given a single ip injection of either CP (200 or 300 mg/kg), MTX (15 mg/kg), or CP (200 mg/kg) plus MTX (15 mg/kg). Controls received an equivalent volume of 0.9% NaCl. On Day 3 after drug treatment, the number of nucleated cells per femur was determined by the method of Fruhman (12). Preliminary experiments showed that the nadir of bone marrow nucleated cellularity occurred on Day 3 after drug treatment.

RESULTS

Effect of Chemotherapy on the Survival of Tumor-Bearing Mice

The relationship between dose and increase in survival times was determined for MTX and CP used individually and in combination in order to determine if a drug schedule could be developed involving a single, simultaneous priming dose of the two drugs, followed by repeated doses of MTX which would result in therapeutic synergism. As can be seen (table 1), in mice bearing a Day-5, L1210 ascites tumor, the combination of CP (200 mg/kg on Day 5) plus MTX (15 mg/kg on Days 5, 7, 9, and 11) produced a mean increase in lifespan (ILS) of 141%. This ILS was greater than that achieved with optimal use of either CP (300 mg/kg on Day 5; 85% ILS) or MTX (30 mg/kg on Days 5, 7, 9, and 11; 84% ILS). This dose schedule fulfills the definition of therapeutic synergism in that the combination was superior to optimal use of either drug alone (13). Presumably, the initial priming dose of CP plus MTX (on Day 5) killed a large fraction of tumor cells, and subsequent doses of MTX (on Days 7, 9,

TABLE 1.—Animal model for demonstrating therapeutic synergism with CP plus MTX in murine ascitic L1210 leukemia*

Group	Dose of CP (mg/kg)	Dose of MTX (mg/kg)	Survival time ± SE (days)	% ILS
1	—	—	7.9 ± 0.35	—
2	200	—	11.4 ± 1.5	44
3	300	—	14.6 ± 1.4	85
4	400	—	10.6 ± 0.9	34
5	—	7.5	11.8 ± 0.9	49
6	—	15	13.2 ± 0.9	67
7	—	30	14.5 ± 0.5	84
8	—	60	13.6 ± 1.1	72
9	200	7.5	17.6 ± 1.3	123
10	200	15	19.0 ± 1.5†	141

*An inoculum of 1×10^{6} L1210 cells was implanted ip into groups of 10 $B6D2F_1$ mice on Day 0. On Day 5 after tumor implantation, mice received ip injections of either CP alone (Day 5), MTX alone (Days 5, 7, 9, and 11), or CP (Day 5) + MTX (Days 5, 7, 9, and 11). Control animals received an equivalent volume of 0.9% NaCl. Animals in both the treatment and control groups were observed for day of death, and mean survival times were determined as described in the Materials and Methods section.

†$P < 0.05$ compared to CP (300 mg/kg) alone by Student's t test.

TABLE 2.—Effect of chemotherapy with either CP, MTX, or CP + MTX on L1210 ascites cell Nos.*

Treatment	Tumor cells $\times 10^{-6}$/mouse†	% of control
Control	56.8 ± 3.7	100
CP (200 mg/kg)	13.1 ± 1.5	23
CP (300 mg/kg)	6.9 ± 0.6	12
MTX (15 mg/kg)	36.4 ± 3.0	64
CP (200 mg/kg) + MTX (15 mg/kg)	4.2 ± 0.4‡	7

*Groups of 4 mice each were treated with a single ip injection of either CP, MTX, or CP + MTX on Day 5 after ip inoculation of 1×10^6 L1210 cells. Control animals received an equivalent volume of 0.9% NaCl. 48 hrs after drug treatment, mice were killed, and the No. of tumor cells in the peritoneal cavity was determined as described in the Materials and Methods section. Tumor cell Nos. from control mice were determined on Day 5 at the time of drug administration to treated mice.

†Values = mean ± SE of 4 mice/group.

‡$P < 0.001$ compared to 200 mg/kg of CP alone and $P < 0.01$ compared to 300 mg/kg of CP alone (Student's t test).

and 11) further decreased the tumor cell population (4,5). Therefore, the combination resulted in a greater ILS than did optimal use of either drug alone.

To examine the contribution of the single, simultaneous dose of CP plus MTX to the resultant therapeutic synergism, we examined some pharmacologic and biochemical effects of single ip injections of either CP, MTX, or CP (200 mg/kg) plus MTX (15 mg/kg) on mice bearing a Day-5, L1210 ascites tumor.

Effect of Chemotherapy on L1210 Ascites Cell Numbers

Tumor cell numbers in the peritoneal cavity of mice treated with either CP, MTX, or CP plus MTX were determined 48 hours after drug treatment. Table 2 shows that CP (200 mg/kg) plus MTX (15 mg/kg) caused a greater decrease in cell numbers than CP (200 mg/kg) alone. In addition, the combination of CP (200 mg/kg) plus MTX (15 mg/kg) caused a greater decrease in cell numbers than the optimal dose of CP (300 mg/kg) alone.

Effect of Chemotherapy on the Incorporation of ^{3}H-dUrd Into DNA of Tumor and Normal Tissues

Knowledge of the magnitude and duration of inhibition of DNA synthesis in tumor, small intestine, and bone marrow after drug treatment might contribute to a better understanding of the effects of the single, simultaneous dose of CP plus MTX as compared to that of either CP or MTX alone on survival of tumor-bearing mice. Therefore, the effects of either CP, MTX, or CP plus MTX on DNA synthesis in tumor and normal host tissues were measured for up to 48 hours after treatment (ie, up to the time just prior to administration of a second dose of MTX). Two and 6 hours after either CP (200 mg/kg), MTX (15 mg/kg), or CP (200 mg/kg) plus MTX (15 mg/kg), the incorporation of ^{3}H-dUrd into DNA of tumor (fig 1A), small intestine (fig 1B), or bone marrow (fig 1C) was markedly inhibited compared to controls. Within 48 hours, recovery of DNA synthesis in small intestine and bone marrow was essentially complete after either CP, MTX, or CP plus MTX. However, tumor DNA synthesis, which had recovered within 48 hours after MTX, still remained strongly inhibited after either CP plus MTX or CP alone. Thus, the time course for recovery of DNA synthesis in normal tissue was similar for either CP, MTX, or CP plus MTX, whereas tumor DNA synthesis still remained inhibited after either CP or CP plus MTX.

Effect of Chemotherapy on Bone Marrow Nucleated Cell Numbers

As one means of evaluating the toxicity of the single, simultaneous dose of CP plus MTX, bone marrow nucleated cellularity was determined in normal (non-tumor-bearing) mice following drug treatment. Bone marrow normally has a large proportion of cells in cycle and thus is particularly sensitive to antiproliferative drugs such as CP or MTX (14). As can be seen in table 3, when the nadir was reached in marrow nucleated cell counts of the mice given CP (200 or 300 mg/kg), MTX (15 mg/kg), or CP (200 mg/kg) plus MTX (15 mg/kg), the number

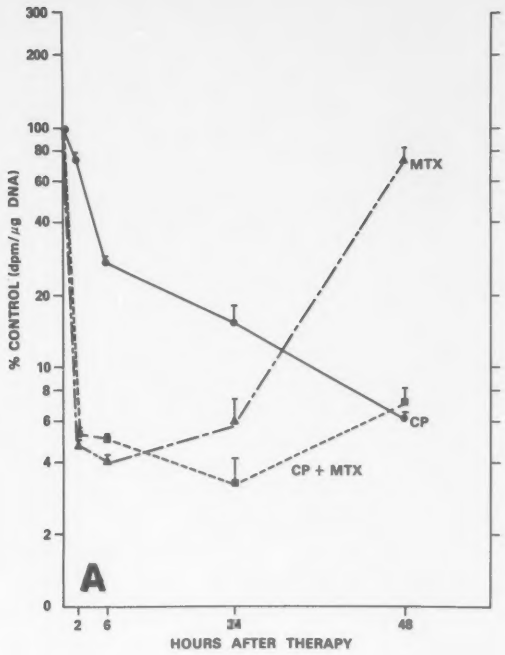
TABLE 3.—Effect of chemotherapy with either CP, MTX, or CP + MTX on the depression in Nos. of nucleated cells/femur of $B6D2F_1$ mice*

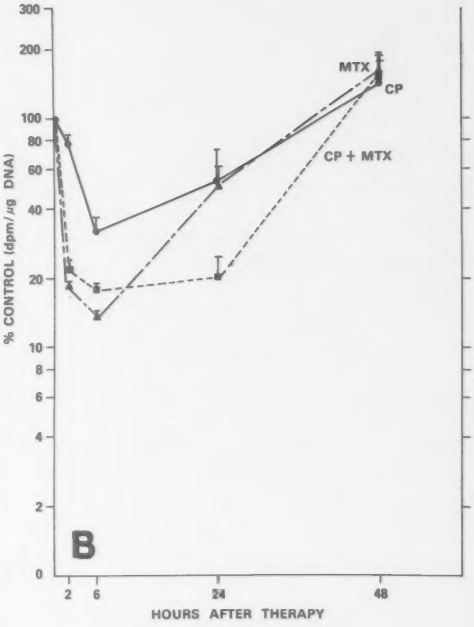
Treatment	Nucleated cells $\times 10^6$/femur†	% of control
Control	19.7 ± 0.86	100
CP (200 mg/kg)	2.19 ± 0.13	11
CP (300 mg/kg)	1.73 ± 0.11	9
MTX (15 mg/kg)	22.9 ± 2.1	116
CP (200 mg/kg) + MTX (15 mg/kg)	2.24 ± 0.08‡	11

*Groups of 4 mice each were treated with a single ip injection of either CP, MTX, or CP + MTX. Control animals received an equivalent volume of 0.9% NaCl. 72 hrs after drug treatment, mice were killed, and the No. of nucleated cells/femur was determined as described in the Materials and Methods section.

†Values obtained for the paired femora of each mouse were averaged and are expressed as mean ± SE of 4 mice/treatment group.

‡$P < 0.02$ compared to 300 mg/kg of CP alone (Student's t test).





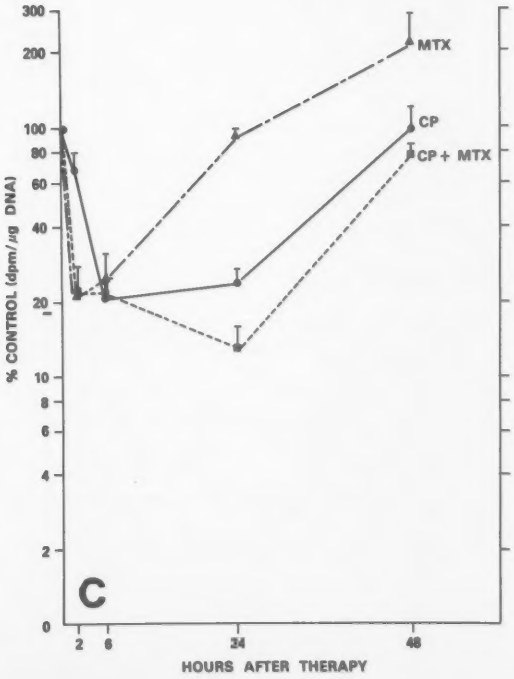


FIGURE 1.—Effect of either CP, MTX, or CP + MTX on the incorporation of ^{3}H-dUrd into DNA of the L1210 ascites tumor (A), small intestine (B), and bone marrow (C). On Day 5 after ip inoculation of 1×10^{6} ascites cells, the mice received either saline (control) or a single ip injection of either CP (200 mg/kg) (●), MTX (15 mg/kg) (▲), or CP (200 mg/kg) + MTX (15 mg/kg) (■). 20 mins before the mice were killed, they received 200 microcuries/240 mg/kg of ^{3}H-dUrd sc. Tumor cells, small intestine, and bone marrow were obtained, and the specific activity of DNA was determined as described in the Materials and Methods section. Each point = mean ± SE of 4 determinations, with 2 mice/determination.

of nucleated cells ± SE averaged 2.19 ± 0.13, 1.73 ± 0.11, 22.9 ± 2.1, and 2.24 ± 0.08 × 10^6 per femur, respectively. The mean number of nucleated cells per femur of mice treated with CP (200 mg/kg) plus MTX (15 mg/kg) was no different than for those treated with CP (200 mg/kg) alone. In addition, the mean number of nucleated cells per femur of mice treated with the optimal dose of CP (300 mg/kg) alone was less than that of mice treated with CP (200 mg/kg) plus MTX (15 mg/kg). These results indicate that the simultaneous dose of CP (200 mg/kg) plus MTX (15 mg/kg) was no more toxic to bone marrow than CP (200 mg/kg) alone and, in addition, that CP (200 mg/kg) plus MTX (15 mg/kg) was less toxic than the optimal dose of CP (300 mg/kg) alone, as determined by the depression in bone marrow nucleated cellularity.

Effect of MTX on the Physiologic Disposition of CP Alkylating Metabolites

It has been shown that the pharmacokinetics of CP in man is altered following treatment with MTX (15). Therefore, it was of interest to determine if the simultaneous administration of CP plus MTX to tumor-bearing mice altered the physiologic disposition of alkylating metabolites of CP in tumor and normal tissues as compared to administration of CP alone. Figure 2 shows the levels of CP alkylating metabolites, as assayed by 4-(*p*-nitrobenzyl)pyridine, in tumors of mice treated on Day 5 after tumor inoculation with either CP (200 mg/kg) plus MTX (15 mg/kg) or CP (200 mg/kg) alone. No statistical differences were observed between the CP- and CP plus MTX-treated groups at any time points in the levels of alkylating metabolites of CP in either tumor (fig 2) or small intestine (not shown). Thus, simultaneous administration of MTX with CP did not alter either the rate of conversion of CP to alkylating metabolites or their persistence in either tumor or small intestine.

Effect of CP on the Physiologic Disposition of MTX

Pretreatment of mice with CP has been shown to alter serum levels of orally administered MTX (16). In order to determine if simultaneous administration of CP plus MTX altered the levels of MTX in either tumor, small intestine, or bone marrow, MTX was measured following treatment of mice with either CP (200 mg/kg) plus MTX (15 mg/kg) or MTX (15 mg/kg) alone on Day 5 after tumor inoculation. Figure 3 shows that concentrations of MTX in tumor were similar at 2, 6, 24, and 48 hours after either CP plus MTX or MTX alone. Although

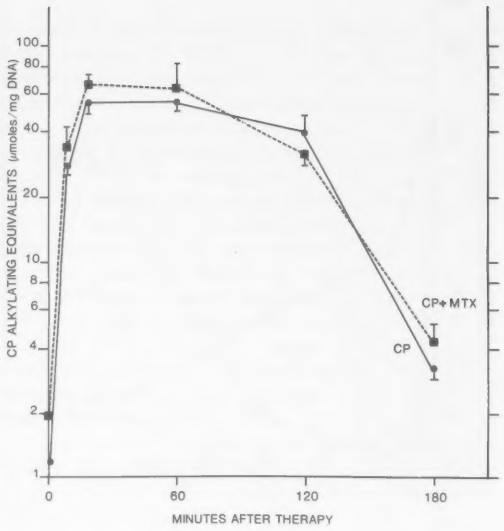


FIGURE 2.—Levels of CP alkylating metabolites in L1210 ascites tumor following treatment of mice with either CP or CP + MTX. On Day 5 after ip inoculation of 1 × 10^6 ascites cells, mice received a single ip injection of either CP (200 mg/kg) (●), or CP (200 mg/kg) + MTX (15 mg/kg) (■). At 10, 20, 60, 120, and 180 mins after drug administration, 4 mice/treatment group were killed, tumor cells were obtained, and alkylating activity was quantitated by colorimetric reaction as described in the Materials and Methods section. Each point = mean ± SE of 4 determinations, with 4 mice/determination.

not shown, similar results were obtained in small intestine and bone marrow. Thus, treatment with a single, simultaneous dose of CP with MTX did not alter the physiologic disposition of MTX in tumor, small intestine, and bone marrow.

DISCUSSION

Therapeutic synergism has been demonstrated in mice bearing a Day-5 ascites L1210 leukemia following administration of CP plus MTX and three subsequent doses of MTX alone. The combination of CP (200 mg/kg on Day 5) plus MTX (15 mg/kg on Days 5, 7, 9, and 11) produced a greater ILS than optimal doses of either CP or MTX alone, so the combination provided a therapeutic advantage over either single agent.

The contribution of the simultaneous dose of CP plus MTX on Day 5 to therapeutic synergism appeared to be related at least in part to its enhanced effect in decreasing tumor cell numbers in the peritoneal cavity without a proportionate increase in toxicity to normal tissue. For example, CP (200 mg/

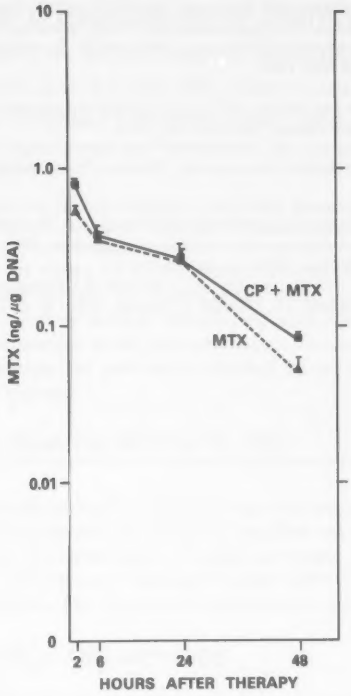


FIGURE 3.—Levels of MTX in L1210 ascites tumor following treatment of mice with either MTX or CP + MTX. On Day 5 after ip inoculation of 1×10^6 ascites cells, mice received a single ip injection of either MTX (15 mg/kg) (▲) or CP (200 mg/kg) + MTX (15 mg/kg) (■). At 2, 6, 24, and 48 hrs after drug administration, mice were killed. Tumor cells were obtained, and MTX content was determined as described in the Materials and Methods section. Each point = mean ± SE of 4 determinations, with 2 mice/determination.

kg) plus MTX (15 mg/kg) treatment on Day 5 after tumor inoculation caused a greater decrease in tumor cell numbers than CP (either 200 or 300 mg/kg) alone. In contrast to the effects on tumor cell numbers, measurements of bone marrow nucleated cellularity showed that CP (200 mg/kg) plus MTX (15 mg/kg) on Day 5 was as toxic as 200 mg/kg of CP alone and less toxic than 300 mg/kg of CP alone.

DNA synthesis in tumor, small intestine, and bone marrow was monitored for 48 hours after injection of a single dose of CP, MTX, or CP plus MTX. The maximum depression of ^{3}H-dUrd incorporation into tumor DNA after 48 hours was comparable for either CP (200 mg/kg) plus MTX (15 mg/kg) or CP (200 mg/kg) alone, although the combination produced a greater inhibition at 2, 6, and 24 hours after drug administration. CP plus MTX or CP alone produced a more sustained inhibition of ^{3}H-dUrd incorporation into tumor DNA than MTX alone. The time course for inhibition and recovery of DNA synthesis in small intestine and bone marrow was similar for all three treatment groups. The enhanced antitumor effect due to the simultaneous administration of CP plus MTX (as measured by the decrease in tumor cell numbers) was no more toxic to normal tissues (as measured by inhibition of ^{3}H-dUrd incorporation into DNA) than either CP or MTX alone.

Alterations in the physiologic disposition of anticancer agents during combination therapy can alter both the action and toxicity of these agents (17). Changes in the hepatic metabolism of CP in mice by other drugs can modify the toxicity of CP (18,19). It appeared that MTX did not change the rate of conversion of CP to alkylating metabolites or their persistence in either tumor or small intestine. Similarly, CP did not appear to alter the physiologic disposition of MTX in either tumor, small intestine, or bone marrow. Thus, the action of CP plus MTX administered simultaneously did not relate to any apparent alteration in their physiologic disposition, as compared to the disposition of either drug administered alone.

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Phase II Evaluation of Dianhydrogalactitol in Lung Cancer: A Southwest Oncology Group Study[1,2]

Brief Reports and Preliminary Communications

Charles D. Haas,[3] Laurence Baker,[4] and Tate Thigpen[5,*]

SUMMARY

A phase II study of dianhydrogalactitol in patients with advanced lung cancer yielded responses in three of 33 patients with adenocarcinoma and in one of 11 patients with large cell anaplastic carcinoma. Small cell and squamous cell carcinomas were unresponsive. Toxic effects were acceptable and were related to serum creatinine values.

[Cancer Treat Rep 65:115–117, 1981]

Dianhydrogalactitol (DAG) is an alkylating derivative of mitolactol (1). Phase I studies established a dose of 30 mg/m²/day × 5 days for phase II trials (2,3). A Southwest Oncology Group (SWOG) phase II trial of DAG in advanced lung cancer is described.

MATERIALS AND METHODS

Seventy-three patients with histologically confirmed lung cancer were registered. Three were not evaluable (one because of a major protocol violation and two because of loss to followup).

Eligibility requirements included signed informed consent, measurable lesions, wbc count > 4000/mm³, platelet count > 150,000/mm³, no prior chemotherapy or radiotherapy within 3 weeks (6 weeks with nitrosoureas), life expectancy of 6 weeks, and no prior DAG or mitolactol.

DAG was given by iv push daily × 5 days every 4 weeks. The starting daily dose for the 45 patients with normal tolerance of prior therapy, no prior nitrosoureas, and normal renal and liver function was 30 mg/m². This was initially reduced to 25 mg/m² for the 21 patients not meeting these criteria. After Feb 27, 1976, four patients with impaired renal function received 15 mg/m² after excessive toxic reactions were noted in a SWOG study of DAG in patients with urologic malignancies. Required studies included cbc and platelet count (weekly during treatment), liver and renal function tests, and lesion measurements. A complete response (CR) was the disappearance of all measurable disease and symptoms for ≥ 4 weeks. A partial response (PR) was ≥ 50% reduction in the sums of the products of the two greatest perpendicular diameters of all measurable lesions for at least 4 weeks. Stable disease included improvement less than a PR or absence of disease progression for at least 4 weeks.

RESULTS

Response data are shown by cell type, performance status (PS), and prior chemotherapy in table 1. One CR lasted 29 weeks in a patient with large cell anaplastic carcinoma, and three PRs lasted 3, 31, and 71 weeks in patients with adenocarcinoma. The patient whose PR was of 3 weeks' duration then died of a bleeding duodenal ulcer without thrombocytopenia. The results are worse if this patient is excluded.

Eleven patients had stable disease lasting 6, 7, 7, 8, 10, 11, 12, 14, 14, 23, and 56 weeks. Forty-four patients had disease progression after one course. Eleven patients died during the first course; none were apparent toxic deaths, and only one patient died of causes other than cancer. Nineteen percent of patients with PS 2–3 and 50% of those with PS 4 died within the first 4 weeks, reflecting the serious illness of these patients and the lack of benefit of DAG.

Toxicity data are summarized in table 2. In all but two instances the worst myelosuppression resulted from the first course of DAG. One drug death occurred in a patient with small cell carcinoma with

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TABLE 1.—DAG in lung cancer response versus cell type, PS,* and prior chemotherapy

Cell type	No. of patients evaluated	% CR + PR†	Prior chemotherapy: Yes — No. of patients (%) with— CR	PR	SD‡	Yes — No. of patients evaluated	No — No. of patients (%) with— CR	PR	SD	No — No. of patients evaluated
Squamous cell										
PS 0–1	5	0	0	0	2	2	0	0	0	3
PS 2–3	6	0	0	0	1	6	—	—	—	—
PS 4	1	0	0	0	0	1	—	—	—	—
Unknown	3	0	0	0	0	3	—	—	—	—
Totals	15	0	0	0	3	12	0	0	0	3
Adenocarcinoma										
PS 0–1	17	12	0	0	2	9	0	2 (25)	2	8
PS 2–3	14	7.1 (7.7)	0	0	1	9	0	1 (20)	1	5
PS 4	1	0	0	0	0	1	—	—	—	—
Unknown	1	0	—	—	—	—	0	0	0	1
Totals	33	9.1 (9.7)	0	0	3	19	0	3 (21)	3	14
Large cell										
PS 0–1	4	25	1 (50)	0	0	2	0	0	0	2
PS 2–3	4	0	0	0	1	2	0	0	0	2
PS 4	2	0	0	0	0	2	—	—	—	—
Unknown	1	0	—	—	—	—	0	0	0	1
Totals	11	9.1 (10)	1 (17)	0	0	6	0	0	0	5
Small cell										
PS 0–1	2	0	0	0	0	2	—	—	—	—
PS 2–3	8	0	0	0	0	7	0	0	0	1
Unknown	1	0	0	0	1	1	—	—	—	—
Totals	11	0	0	0	1	10	0	0	0	1
All cell types										
PS 0–1	28	10.7 (11.1)	1 (7)	0	4	15	0	2 (15)	2	13
PS 2–3	32	3.1 (3.8)	0	0	3	24	0	1 (12)	1	8
PS 4	4	0	0	0	0	4	—	—	—	—
Unknown	6	0	0	0	1	4	0	0	0	2
Totals	70	5.7 (6.8)	1 (2)	0	8	47	0	3 (13)	3	23

*SWOG scale.
†% in parentheses excludes patients with early deaths.
‡SD = stable disease.

TABLE 2.—DAG toxicity (25–30 mg/m²/day × 5 days)

	All evaluable patients		Patients with serum creatinine < 1.0 mg/ml		Patients with serum creatinine 1.0–1.9 mg/ml	
	No.	%	No.	%	No.	%
Wbcs × 10^3/mm³						
≥ 4.0	32	52	12	50	17	52
3.0–3.9	8	13	5	21	3	9
2.0–2.9	12	20	5	21	7	21
1.0–1.9	7	11	2	8	4	12
< 1.0	2	3	0	—	2	6
Platelets × 10^3/mm³						
≥ 150	24	39	12	50	12	36
75–149	16	26	6	25	8	24
50–74.9	9	15	4	16	4	12
25–49.9	5	8	2	8	2	6
< 25	7	11	0	—	7	21

marrow involvement, heavy prior therapy, and a serum creatinine of 1.2 mg/ml. The incidences of severe leukopenia (< 2000 cells/mm^3) and thrombocytopenia (< 50,000 platelets/mm^3) were each 8% for patients with serum creatinine values < 1.0 mg/ml and were 18% and 27%, respectively, for patients with creatinine values of 1.0–1.9 mg/ml. The only patient whose serum creatinine was > 2.0 mg/ml (2.3) had no toxic reaction at 15 mg/m^2/day × 5 days.

Nausea and vomiting were rare. Three instances of skin ulceration after extravasation were noted.

DISCUSSION

Analysis by cell type, pretreatment PS, and presence or absence of prior chemotherapy identifies no sizeable subgroup with a strong likelihood of response. DAG activity was marginal in another study (4). SWOG plans no further evaluations of this drug in lung cancer.

Toxic reactions are similar to those previously reported (2–4). Thrombocytopenia is the most serious effect and is related to serum creatinine values.

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Phase II Study of Spirogermanium in Advanced Ovarian Malignancy[1,2]

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SUMMARY

In the present phase II trial 18 heavily pretreated patients with advanced ovarian malignancy were treated with spirogermanium, 11 im and seven iv. Objective responses (partial remission) were obtained in two (11%) of the 18 patients, with a remission duration of 5 and 23+ months, respectively. An additional four (22%) patients had stable disease. Each of these six patients had a better quality of life. There were few side effects. Myelosuppression, nausea, or other gastrointestinal side effects did not occur. The present investigation shows that spirogermanium is useful as a palliative chemotherapeutic agent in patients with advanced ovarian malignancy.

[Cancer Treat Rep 65:119–120, 1981]

Spirogermanium belongs to the azaspiranes, which constitute a new class of cytotoxic agents. It inhibits protein synthesis in vitro, while the synthesis of DNA and RNA in vitro is initially unchanged (1). Spirogermanium has a cytotoxic effect on HeLa cells and on cells from human malignant lymphoma in vitro and moderately inhibits the growth of mammary carcinoma 13762 and cancer prostatae 11095 in rats.[6] The lifespan of rats transplanted with Walker 256 carcinosarcoma was increased (1). Toxicity studies in dogs and mice treated with single im and iv doses and in rats receiving im therapy for 6 months showed toxic effects in the CNS, gastrointestinal tract, liver, kidney, erythrocytes, and lymphoid tissue.[6]

In a pilot study including 11 patients, an objective response was observed in various types of malignancies when the drug was given im or iv at doses ranging from 40 to 120 mg/m².[6]

In our phase I study, in which the iv doses successively were increased from 20 to 140 mg/m² twice weekly, the dose-limiting factors have been lethargy and severe paresthesia. Other symptoms were dizziness and a feeling of drowsiness. Otherwise, no side effects were observed. In particular, no depression of leukocytes, lymphocytes, or platelets occurred (2).

The few, but promising, clinical data and the low frequency of side effects prompted us to perform a phase II study in advanced and recurrent ovarian malignancy.

MATERIALS AND METHODS

Eighteen patients with advanced ovarian malignancy fulfilling the following criteria were included in the study: wbc count $\geq 2.0 \times 10^9$/liter, platelet count $\geq 75 \times 10^9$/liter, creatinine ≤ 115 μmols/liter, measurable tumor, and recurrent disease resistant to either radiotherapy or chemotherapy or both.

All patients had previously had some kind of surgery: five had had salpingo-oophorectomy-hysterectomy; seven had had salpingo-oophorectomy; and six had had explorative laparotomy. Prior to spirogermanium treatment, three patients had received chemotherapy, one had had radiotherapy, and 14 had received both types of therapy for relapse.

Histologic examination showed serous adenocarcinoma in seven patients, mucinous adenocarcinoma in two, poorly differentiated adenocarcinoma in

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[2]Spirogermanium (S-99A, Spiro-32) was supplied by Unimed Inc, Somerville, NJ.

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[6]Rice LM, Slavik M, and Schein P. Clinical brochure: spirogermanium (NSC-192965). Prepared by the National Cancer Institute, Bethesda, MD, 1977.

seven, and granulosa cell cancer in two. Before spirogermanium treatment was started and regularly during therapy, the extent of the disease was assessed by physical and gynecologic examination, chest roentgenogram, isotope scan of the liver, and fine-needle aspiration biopsy of available metastatic lesions in the pelvis minor, lymph nodes, lungs, pleurae, and liver. When indicated, the examinations were supplemented by ultrasonic examination, computerized tomography of the abdomen, and various isotope scans. Routine blood examinations were regularly performed.

Spirogermanium was administered twice weekly at a dose of 50 mg/m² either im (11 patients) or by iv drip infusion during 15–30 minutes (seven patients). All patients were treated for at least 4 weeks. Patients with stable disease were then treated until progressive disease occurred and patients with partial remission were treated until relapse.

The results of treatment are defined as follows: *complete remission (CR)*—the complete disappearance of all measurable lesions for at least 1 month; *partial remission (PR)*—at least a 50% reduction of measurable tumor with no pleural or ascitic fluids persisting; *stable disease*—a 0–49% decrease of measurable tumor volume and no new lesions, or improvement in nonevaluable lesions such as ascitic and pleural fluids (ascitic and pleural fluids might persist, but may not increase, and an increase in the time intervals between thoracocentesis and laparocentesis must have occurred); *progressive disease*—an increasing volume of tumor or new lesions during treatment without remission or shortening of time intervals between paracenteses; and *relapse*—after remission, a new lesion or recurrence in a previously regressed area.

RESULTS

Of 11 patients receiving im spirogermanium, one achieved PR (23+ months) and three had stable disease (2, 3, and 18+ months). With iv administration, one patient achieved PR (5 months) and one had stable disease (4 months). The median survival time of the patients with progressive disease was 3 months (range, 1.5–5).

An objective response was observed in local recurrent tumor (verified by palpation and ultrasound examination) and in biopsy-confirmed metastases in the liver (verified by palpation, liver scan, ultrasound examination, and normalization of laboratory examinations). All of the six patients achieving PR or having stable disease had a better quality of life.

Despite previous chemotherapy, an objective remission was obtained in one histologically determined granulosa cell cancer and in one histologically determined poorly differentiated adenocarcinoma. Thirteen of the 16 patients who died were autopsied and no macroscopic or microscopic pathologic changes secondary to therapy could be discriminated.

Spirogermanium did not produce any myelosuppression, nausea, or other gastrointestinal side effects. Extreme tiredness occurred in five patients, reversible paresthesias occurred in three, and dizziness lasting for 4–6 hours occurred in three. After im dosing, six patients complained of severe pain at the injection site and two of them developed a sterile abscess. No local complications were observed after iv administration.

DISCUSSION

Treatment with spirogermanium is well-tolerated and induces an objective response in ovarian malignancy. As second-line chemotherapy, the overall response rate was 11%; this favorably compares with the data of Stanhope et al (3) and Piver et al (4) while it was about the same as the response rate obtained by Johnsson et al (5). There are few side effects with spirogermanium and it can be administered over a long time without any toxicity. Because of its lack of myelosuppression and since objective responses were seen in patients with advanced disease, spirogermanium might be a useful chemotherapeutic agent in patients with ovarian malignancy.

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Phase II Clinical Trial of Dianhydrogalactitol for Remission Induction in Children With Acute Leukemia: A Southwest Oncology Group Study[1,2]

Brief Reports and Preliminary Communications

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Dianhydrogalactitol (DAG) is the major conversion reaction product of the cytotoxic agents mitolactol (DBD) and dibromomannitol (DBM) (1,2). DAG produces greater inhibitions of DNA, RNA, and protein synthesis and is more effective in cross-linking DNA than DBD or DBM.

Antitumor activity of DAG was seen in L1210 and P388 leukemias, myeloid ascites leukemia induced by 7,12-dimethylbenz[*a*]anthracene, and transplanted myeloid leukemia. Activity was also seen in a variety of mouse and rat solid tumors (3). The drug's non-cell-cycle-dependent activity suggested potential activity in human neoplasms.

Independent phase I studies using DAG in five consecutive daily doses every 4 weeks suggested a dose of 30 mg/m²/day × 5 for phase II studies (4) (Hoogstraten et al, personal communication). Dose-limiting toxicity was myelosuppression. Thrombocytopenia was more frequent than neutropenia, with nadir cell counts occurring at a median of 21 days (range, 15–22) and recovery in 7–10 days. Mild nausea and vomiting was observed. No significant renal or hepatic toxicity was noticed. Antitumor activity occurred in renal, bladder, and small cell lung cancers.

The present study was designed: (*a*) to ascertain the effectiveness of DAG in late-stage acute leukemia of children; (*b*) to further observe the toxic effects of DAG in children; and (*c*) to determine any factors contributing to these toxic effects.

MATERIALS AND METHODS

All children < 18 years of age with late-stage acute lymphatic leukemia and with proven bone marrow relapse (> 25% blast cells in the bone marrow) were eligible for the study. Patients were resistant to treatment with vincristine, prednisone, 6-mercaptopurine, methotrexate, cyclophosphamide, L-asparaginase, and doxorubicin. Prior to initiating therapy with DAG, cbc, platelet count, serum BUN, creatinine, bilirubin, SGOT, SGPT, and creatinine clearance were obtained. These studies were repeated in the third and fourth weeks of therapy. Bone marrow examination was required on Days 0 and 28. To be eligible for analysis, patients were required to have at least one full course of DAG and to have a minimum survival of 3 weeks after completion of the course. DAG was administered at a dose of 30 mg/m²/day × 5. Patients with evidence of hepatic toxicity (bilirubin > 2 mg/100 ml) received 25 mg/m²/day × 5, and those with only one functioning kidney or with impaired renal function (creatinine clearance < 65 ml/minute) received 15 mg/m²/day × 5.

RESULTS AND DISCUSSION

Five of the 27 children entered in the study had an early death and were not considered in study analysis. For three of the five patients, hemorrhage and infection were the causes of death, and in two of the five, cause of death could not be determined, since they died at home. All five children had progressive disease at the time of death. Twenty-two of the 27 patients were evaluable.

Fourteen children received the full dose of DAG (30 mg/m²); ten received two or more courses, while 12 had only one course.

The mean number of prior bone marrow inductions in these children ranged from two to nine (mean, 6.2 inductions). Analysis of prognostic factors revealed that 15 of 22 patients were in the favorable age group (2–10 years) at the time of initial diagnosis, while seven were in the unfavorable age group. Of the 22 children, six had wbc counts

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> 10,000/mm³ and seven had massive organomegaly at the time of diagnosis. Therefore, about one half of the children were originally in the good-risk category.

The major toxicity was hematopoietic, with thrombocytopenia being more common than neutropenia. Toxicity of this nature is hard to ascertain in a patient with bone marrow disease but in 14 of 22 patients platelet counts dropped to < 25,000/mm³ and wbc counts to 1000/mm³. Nadir cell counts occurred around Day 14. Gastrointestinal toxicity was mild. Nausea and/or occasional vomiting occurred in only three of 22 patients. No therapeutic response, either complete or partial, was noticed in any patient.

With this dose schedule, DAG does not show activity in children with advanced end-stage acute lymphatic leukemia. Major toxic effects were severe thrombocytopenia and neutropenia, with anemia being relatively rare. Gastrointestinal toxicity was mild, and no hepatic or renal toxicity was noticed. The prognostic factors did not seem to affect the outcome of the therapeutic response.

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5-FU in the Treatment of Small Cell Anaplastic Carcinoma of the Lung: A Phase II Trial[1,2]

Brief Reports and Preliminary Communications

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SUMMARY

The effect of 5-FU as a single agent in small cell anaplastic carcinoma of the lung was evaluated in 26 patients. All patients had received prior chemotherapy. Ten patients were treated with 5-FU doses of 600 mg/m² iv on Days 1–5, followed by 1000 mg/m² iv on Day 22 and then every second week. Among eight evaluable patients, partial remissions of 42–45 days' duration were seen in three cases. However, toxic reactions, consisting of thrombocytopenia (< 50,000/mm³) in five cases and leukopenia (< 1000/mm³) with septicemia in two patients, were unacceptable. Therefore, the remaining 16 patients were treated with 400 mg/m² iv daily for 5 days, while the maintenance dosage of 5-FU remained unchanged. With this dosage the hematologic toxicity was moderate, but no responses were observed. It is concluded that 5-FU has a low order of activity in previously treated patients with small cell anaplastic carcinoma of the lung.

[Cancer Treat Rep 65:123–125, 1981]

Small cell anaplastic carcinoma of the lung is the histologic type of lung cancer most sensitive to cytotoxic agents. Using combination chemotherapy, objective regression can be obtained in 80%–85% of all patients, resulting in significant prolongation of the median survival (1). In spite of the high remission rate, the duration of response is generally short, presumably because of development of resistance to the drugs used. Additional drugs effective against this type of lung cancer are therefore urgently needed.

By a review of the literature it was apparent that 5-FU has not been adequately tested as a single agent in small cell anaplastic carcinoma, in spite of its use in combination chemotherapy (2,3). The present study was therefore undertaken to examine the activity of 5-FU in this disease.

PATIENTS AND METHODS

Twenty-six patients with histologically verified small cell anaplastic carcinoma of the lung were studied. All patients had received prior chemotherapy, but none of them had previously received 5-FU. No chemotherapy was given during the last 14 days before entering the study (for the nitrosoureas, an interval of 6 weeks was required). Wbc and platelet counts were ≥ 4000/mm³ and ≥ 100,000/mm³, respectively. All patients had measurable or evaluable disease, and informed consent was obtained before entering the trial.

Minimal pretreatment investigations included physical examination with evaluation of performance status; Hgb and wbc and platelet counts; LDH, alkaline phosphatase, SGOT, and creatinine levels; and chest roentgenogram. Hgb and wbc and platelet counts were repeated at least every 2 weeks, and the other investigations were repeated every 4 weeks.

Tumor regression was defined as a ≥ 50% decrease in the product of the longest and widest diameters of measurable tumors or a ≥ 75% decrease in evaluable but not clearly measurable tumors, without the appearance of new lesions. Progression was defined as a > 25% increase in the product of the longest and widest perpendicular diameters of measurable tumors or a > 50% increase in evaluable but not clearly measurable tumors. The appearance of new lesions was similarly considered as progression of the disease. Because of uncertainty con-

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cerning the penetration of 5-FU across the blood-brain barrier, progression within the central nervous system was not accepted as a sign of progression of the disease.

In the first ten patients, 5-FU was given in a dose of 600 mg/m² iv on Days 1–5 followed by 1000 mg/m² iv on Day 22 and repeated every 2 weeks. Because of severe hematologic toxicity, the initial dosage was subsequently decreased to 400 mg/m² iv on Days 1–5 in the remaining 16 patients. Maintenance dosage was unchanged.

The rules for dose modifications are summarized in table 1.

TABLE 1.—Dose reduction of 5-FU related to hematologic toxicity

Wbcs/mm³	Thrombocytes/mm³	Dose (%)
> 4000	> 100,000	100
3000–3999	75,000–99,000	67
2000–2999	50,000–74,000	33
< 2000	< 50,000	0

RESULTS

Ten of the 26 patients included in the study received the high initial dose of 600 mg/m² of 5-FU. Nine were males, and one was a female. Median age was 59 years (range: 53–72). All patients except one had received previous treatment with at least four of the following drugs: CCNU, methotrexate, cyclophosphamide, vincristine, doxorubicin, VP-16-213, and cisplatin. One patient, a man aged 72 years, had received only prednimustine before entering the study. In addition, one patient had received local irradiation. Two patients died < 2 weeks from start of treatment and were not evaluable.

The median duration of treatment in the remaining eight patients was 49 days (range: 21–56). Three of these patients obtained partial remissions of 42, 45, and 45 days' duration, with the response occurring in the primary tumor in two patients and at the metastatic site in one patient. One of the responders was the patient who had previously been treated with prednimustine. Toxic effects were severe and included thrombocytopenia (< 50,000/mm³) in five cases complicated by severe bleeding in one patient. Two patients had wbc counts < 1000/mm³ and septicemia. A fatal hematologic toxic reaction was observed in one patient. Three patients had severe stomatitis, while other side effects consisted of mild nausea and vomiting.

Sixteen patients received the lower initial dose of 400 mg/m² of 5-FU. Eleven patients were males, and five were females. The median age was 63 years (range: 25–74). These patients had all been treated with at least four of the following drugs: CCNU, methotrexate, cyclophosphamide, vincristine, doxorubicin, VP-16-213, and vindesine. Four patients had received mediastinal irradiation. The median duration of treatment with 5-FU was 21 days (range: 21–56).

Among the 16 patients receiving the reduced initial dose of 5-FU, one patient died with progression of the disease within 2 weeks after entering the study. None of the other 15 evaluable patients showed any response.

The toxic effects were milder than with the high initial dose of 5-FU. Three patients developed thrombocytopenia < 50,000 platelets/mm³, complicated by bleeding in one case. Three patients had leukopenia, leading to septicemia in one case. Moderate stomatitis, diarrhea, nausea, and vomiting were observed in three patients.

DISCUSSION

5-FU has been in clinical use since 1952, but apparently it has never been thoroughly evaluated in small cell anaplastic carcinoma of the lung. In a study comparing cyclophosphamide and 5-FU in a group of patients with lung cancer of mixed histology, Brugarolas et al (4) treated six patients with small cell carcinoma with 5-FU alone without observing any response.

In our study, ten patients received a high initial dose of 5-FU. Three remissions of brief duration were noted, but toxicity was unacceptable. No response was observed in the remaining 16 patients treated with a lower initial dose of 5-FU, in spite of moderate drug-induced toxic effects. An apparent dose-response relationship of 5-FU in the treatment of small cell anaplastic carcinoma was thus observed, similar to observations for breast cancer.

However, our study failed to demonstrate any activity of 5-FU in doses that do not give rise to excessive toxic reactions when given to patients with small cell carcinoma who are resistant to standard chemotherapy. Clearly, the effect of an anticancer drug may be underestimated and toxicity may be aggravated as a result of heavy prior treatment, and our data do not permit any firm statement concerning the activity of 5-FU in untreated patients. However, considerable activity has been demonstrated for other drugs, eg, VP-16-213 and vindesine, when studied under similar circumstances (5,6). Compared to such compounds, 5-FU seems to deserve little interest for inclusion in future combination regimens.

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Phase II Evaluation of Metoprine in Patients With Advanced Colorectal Carcinoma[1,2,3]

Brief Reports and Preliminary Communications

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Metoprine is an inhibitor of dihydrofolate reductase and is highly lipid-soluble (1–3). In animal systems, considerable activity has been demonstrated against the Ehrlich ascites carcinoma, Sarcoma 180 in BD2F, mice, leukemia in mice, and Walker carcinosarcoma 256 (2,4). Studies in man have shown responses in lung carcinoma, hypernephroma, bladder carcinoma, mycosis fungoides, acute leukemia, CNS leukemia, and epidermoid carcinoma of the anus (5,6). Toxic effects of phase I trials of metoprine included leukopenia, thrombocytopenia, megaloblastoid changes, nausea and vomiting, mucositis, rash, and mental confusion.

A phase II trial of metoprine was conducted between June and November 1979. A low-dose schedule of metoprine without leucovorin rescue was used because of its ease of administration and its convenience for the patient.

MATERIALS AND METHODS

Twenty-eight patients with histologically confirmed measurable metastatic colorectal carcinoma were entered in the study. Metoprine was given in tablet form as a single oral dose. Doses were repeated at intervals of 2 weeks. The initial dose of metoprine, derived from previous phase I studies at Memorial Sloan-Kettering Cancer Center, was 65 mg/m². This was later lowered to 50 mg/m² due to unacceptable toxicity at the higher dose. In the absence of thrombocytopenia or significant toxicity after two cycles of therapy, doses were escalated by 5 mg/m². If platelet counts were $< 100 \times 10^3$/mm³ and/or wbc counts were $< 2.5 \times 10^3$/mm³ after a cycle of therapy, subsequent doses were attenuated by 10 mg/m². Leucovorin rescue was used only if the platelet count fell to $< 30 \times 10^3$/mm³. Leucovorin rescue was given as an initial 50 mg iv bolus; the patient then received 50 mg orally in liquid form every 12 hours for five subsequent doses.

A complete response was defined as the complete disappearance of all evidence of disease. A partial response was defined as a > 50% decrease in the sums of the products of the largest perpendicular diameters of all measurable tumors for at least 1 month. A minor response was a > 25% decrease in measurable disease. An adequate trial required that the patient receive at least three courses of therapy with toxic reactions.

The median age of the patients was 54.5 years (range, 36–74 years), and the median Karnofsky performance status was 80 (range, 60–100). Sites of measurable metastatic disease included lung (61%), liver (50%), rectum (7%), pelvis (7%), abdomen (7%), and lymph nodes (7%). The median time from diagnosis of metastatic disease to entry in this study was 10 months (range, 1–36 months). Twenty-three patients had received prior chemotherapy, and four had received prior radiotherapy as well. Prior chemotherapy consisted of 5-FU in 93% of the patients, methyl-CCNU in 86%, streptozocin in 54%, and other phase II chemotherapeutic agents in 21%.

RESULTS

Twenty-four of the 28 patients who entered the study received an adequate trial. Of the four inadequately treated patients, one died within 2 weeks from progressive disease, two required interruption of therapy for radiation treatment (pelvis and brain, respectively), and one patient was lost to followup.

Two of the 24 evaluable patients (8%) had partial responses. One patient who had failed three previous drug trials had a > 75% reduction in lung metastases for 6+ months. The other patient, also pretreated with chemotherapy, had a > 50% reduction in lung metastases. Both of these patients

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[3]Metoprine was supplied by Burroughs-Wellcome, Research Triangle Park, NC.

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[5]We acknowledge the technical assistance of Ms. Jane Howard and the secretarial assistance of Ms. Cathie Cassidy.

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responded by the end of three treatments. Six patients had stable disease for a median of 3 months (range, 2.5–5 months), and 16 had clear disease progression.

The chief toxic effect encountered in this study was thrombocytopenia, which occurred in 23 patients (82%) and was dose-limiting. The median platelet count nadir was 79 × 10^3/mm^3. Twelve patients had platelet counts < 50 × 10^3/mm^3. Eight patients had bleeding episodes requiring platelet transfusions and leucovorin rescue; five patients required hospitalization. Six (66%) of the nine patients who entered at a dose schedule of 65 mg/m^2 had platelet counts < 50,000/mm^3; three (33%) had bleeding episodes. The three patients who had platelet counts < 20,000/mm^3 with the high dose also dropped to this level with the low dose. Six (31%) of the 19 patients who started at 50 mg/m^2 had platelet counts < 50,000/mm^3; two patients had bleeding episodes requiring platelet transfusions and leucovorin rescue. Within 6 days of administration of leucovorin rescue, all patients had platelet count elevations (median, 180 × 10^3/mm^3; range, 65–345 × 10^3/mm^3) and no further bleeding. The degree of thrombocytopenia was unrelated to creatinine, bilirubin, or alkaline phosphatase. The median wbc count nadir in the study was 3.7 × 10^3/mm^3 (range, 0.9–12.8/mm^3). Only four patients (14%) had wbc count nadirs < 2 × 10^3/mm^3; three of these had received the 65 mg/m^2 dose. Within 5 days, the wbc count was elevated in all patients, and there were no episodes of drug-related sepsis.

Nonhematologic toxicity included vomiting (18%), nausea alone (18%), mucositis (11%), fatigue (35%), and rash (18%). The rash was erythematous and maculopapular with a predilection for the truncal areas; it was self-limited. Two patients developed increased pigmentation of sun-exposed areas.

DISCUSSION

Two of 24 adequately treated patients with advanced colorectal cancer had a significant response to metoprine, giving a response rate of 8% (range, 1.5%–24%; $P < 0.05$). This response rate was observed in the face of substantial toxicity, chiefly in the form of thrombocytopenia, in 82% of the patients. Toxicity was unpredictable at a given dose of metoprine and did not correlate with renal or hepatic function. Furthermore, toxicity was cumulative, with individual patients experiencing side effects at doses that they previously tolerated without difficulty.

The two responding patients in our study were both heavily pretreated patients with lung metastases. Each patient initially received 65 mg/m^2, a dose which was later attenuated due to toxicity. In spite of progressively lowering the dose, the responses in both patients are ongoing. We conclude that doses of metoprine that are tolerable without scheduled leucovorin rescue display minor evidence of antitumor activity in heavily pretreated patients with advanced colorectal cancer. Further studies of high-dose metoprine with leucovorin rescue are in progress.

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Phase II Trial of Vindesine in Patients With Small Cell Lung Carcinoma[1,2]

R. B. Natale, R. J. Gralla, and R. E. Wittes[3,4,*]

Vindesine is a semisynthetic vinca alkaloid similar to vinblastine in its distribution and metabolism (1) but more closely resembling vincristine in its activity against murine hematologic and solid tumor models (2). In phase II trials vindesine has shown activity against a number of human solid tumor (3–6) and hematologic (7,8) malignancies. Both vincristine and VP-16-213 (another mitotic spindle inhibitor) have well-established activity in small cell lung carcinoma (SCLC) (9,10), and an objective response in a patient with SCLC was observed in an early clinical trial with vindesine (11). We therefore conducted a phase II trial of vindesine in patients with SCLC at the Memorial Sloan-Kettering Cancer Center.

MATERIALS AND METHODS

Twenty-six patients with objectively measurable and histologically confirmed SCLC were entered in the study. Before patients received vindesine, the requirements included a wbc count $\geq 4000/mm^3$, a platelet count $\geq 120{,}000/mm^3$, no treatment for at least the preceding 3 weeks, and informed consent. At the start of therapy all patients had a complete medical history, physical examination, and laboratory evaluation including cbc, 12-channel biochemical profile, serum electrolytes and creatinine, 5'-nucleotidase, and chest roentgenogram. Radionuclide liver scans were obtained in patients with hepatomegaly and abnormal biochemical liver function tests, and bone scans were obtained in patients with suspected bone metastases. Each patient had a weekly physical examination and cbc and a monthly evaluation of chest roentgenogram, unless indicated at an earlier date.

Vindesine (supplied by Eli Lilly and Co, Indianapolis, IN) was administered weekly by rapid injection through an established free-flowing iv line. The initial dose was 3.0 mg/m^2, which was increased by 0.5 mg/m^2 each week (but not exceeding 4.0 mg/m^2/week, total dose) in the absence of dose-limiting toxicity. The dose was attenuated by 50% if the wbc count was 2000–2999/mm^3 or if the platelet count was 75,000–100,000/mm^3; chemotherapy was withheld for at least 1 week if blood cell counts were below these levels.

An adequate trial required a minimum of three weekly doses of vindesine and at least 2 weeks' survival. Therapeutic response criteria were defined as: *partial remission (PR)*—a $\geq$ 50% decrease of all measurable lesions for $>$ 1 month; *minor response*—an objective tumor decrease of $\geq$ 25% but less than that required for a PR; and *disease progression*—an objective increase in measurable disease or the appearance of new tumor lesions.

Patient Characteristics

There were 15 males and 11 females with a median age of 58 years (range, 41–72) and a median Karnofsky performance status of 60 (range, 40–90). The median time from diagnosis until entry in this study was 11 months (range, 3–16.5). All patients had received prior combination chemotherapy including cyclophosphamide (100%), doxorubicin (96%), vincristine (96%), VP-16-213 (70%), cisplatin (70%), methotrexate (46%), a nitrosourea (46%), and procarbazine (30%). Most patients had also received prior radiation therapy to whole brain (96%) and mediastinum (50%). Eighteen patients (70%) had more than one site of tumor involvement, including lung (26 patients), lymph nodes (11), liver (six), bone (nine), and iris (one).

RESULTS

Major objective responses (PRs) occurred in five of 24 adequately treated patients, with an observed

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TABLE 1.—Toxicity of vindesine

Toxic effects	No. of patients (%)
Neurotoxicity	10 (42)
Constipation	7 (29)
Nausea and/or vomiting	7 (29)
Fever	1 (4)
Wbc count nadir (cells/mm^3)	
≥ 4000	3 (12)
3000–3999	6 (25)
2000–2999	11 (46)
< 2000	4 (16)
Platelet count nadir (cells/mm^3)	
≥ 200,000	10 (42)
150,000–199,999	8 (33)
100,000–149,999	4 (16)
< 100,000	2 (8)

response rate of 21% (95% confidence interval, 9%–40%) and a median duration of response of 2.0 months (range, 1.5–5). One additional patient experienced a minor response. Trials were considered inadequate in two patients because they received only one dose of vindesine each and died from progressive disease on Days 10 and 13 from the start of the protocol. Four of the five patients achieving PR had more than one site of measurable tumor including lung (five patients), lymph nodes (two), liver (two), and iris (one) (12). Comparison of age, sex, performance status, time from diagnosis to start of the vindesine trial, and total doses of previous vincristine or VP-16-213 showed no significant differences between responders and nonresponders. Twenty-four patients have now died of their disease. The median time from entry in this study to death was 13 weeks (range, 1.4–38).

The median number of doses given was six per patient (range, one to 22 doses), and dose escalation was possible in 50% of patients. Leukopenia was the most common dose-limiting toxic effect (table 1) but was not severe and did not result in any episodes of sepsis (median wbc count nadir, 2600/mm^3; range, 800–6900/mm^3). As observed in previous vindesine trials, thrombocytopenia was unusual and rarely dose-limiting.

Neurotoxicity, manifested by paresthesias, decrease in deep tendon reflexes, myalgias, muscle weakness, or constipation, was common (table 1) but well-tolerated; it was not dose-limiting and did not require dose attenuation. Severe neuropathy, resulting in marked muscle weakness or a decrease in manual dexterity or in ambulatory ability, was not observed. Other less common toxic effects were mild and not dose-limiting (table 1). Alopecia, common with vindesine, could not be evaluated in this study because of pre-existing alopecia from previous therapy.

DISCUSSION

Because of the heavy extent of prior therapy in these patients, the activity of vindesine seems noteworthy. The brevity of the responses, however, limits the clinical utility of this agent, at least in pretreated patients. The extent of prior treatment with vincristine did not appear to affect response to vindesine. We cannot conclude that vindesine has no cross-resistance with vincristine since the latter agent was always given as part of combination regimens in which the cumulative dose was substantially lower than it would have been if it were being given as a single agent. Questions regarding the relative activities, toxic effects, and cross-resistance patterns of these two vinca alkaloids would be best investigated by a randomized comparison using a crossover design. Such a study is obviously not possible when vincristine is used as part of primary therapy, as in our institution.

Optimization of doses and schedules of administration of the many available drugs in SCLC and identification of new active agents remain our priorities in the treatment of this disease. Vindesine is an active agent in SCLC and its place in current therapy deserves further definition.

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Phase II Trial of AMSA in Patients With Refractory Small Cell Carcinoma of the Lung[1]

Brief Reports and Preliminary Communications

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Once patients with small cell carcinoma of the lung (SCCL) relapse from primary therapy, the number of active agents that can be used in order to produce secondary responses is limited. It is therefore highly desirable to identify activity among new agents as potential second-line therapy for patients failing initial combination chemotherapy. The purpose of the current phase II study was to test the therapeutic efficacy of AMSA, a new acridine derivative (1–3) which through intercalation and external binding to DNA exerts antitumor activity in patients with SCCL who had failed conventional therapy (4–7).

MATERIALS AND METHODS

Patients with histologically confirmed SCCL and progressive disease after conventional therapy were included in the study. Eligibility criteria included a measurable lesion, an anticipated survival of at least 8 weeks, and complete recovery from toxicity due to previous therapy. Before treatment, patients were evaluated with cbc, platelet count, and liver and renal function tests. Pretreatment bone scan, bone survey, bone marrow biopsy, brain scan (or computerized tomography brain scan), and whole-lung tomography were performed as clinically indicated.

Seventeen patients with SCCL refractory to conventional therapy were treated with AMSA. There were ten males and seven females, with a median age of 61.3 years (range, 49–76). Prior therapy included alternating combination chemotherapy with the following drug regimens: cyclophosphamide, doxorubicin, and VP-16-213; and CCNU, vincristine, methotrexate, and procarbazine (6). Six of the 17 patients had also received palliative irradiation to intrathoracic sites of disease. Measurable parameters in 17 patients treated with AMSA consisted of a chest lesion in 14, enlarged peripheral lymph nodes in six, subcutaneous metastases in two, and liver metastases in eight. Furthermore, five patients had a positive bone marrow examination. All but two patients had involvement of two or more metastatic sites.

AMSA was administered iv at an initial dose of 120 mg/m^2 every 3 weeks. Therapy was continued until there was evidence of disease progression. Dosage modifications were based on the blood cell counts on Day 21. No dose modifications were made for hematologic nadirs if no hemorrhagic or infection problem had occurred. If the wbc count was $> 4000/\mu l$ and the platelet count was $> 150{,}000/\mu l$, the dose of AMSA was escalated by 20 mg/m^2. If the wbc count was 3000–4000/μl and the platelet count was 100,000–150,000/μl, the AMSA dose was unchanged. Blood cell counts below this necessitated a delay of 1 week and, if full recovery had still not occurred, a bone marrow aspirate and biopsy was performed to rule out bone marrow invasion with tumor. If the biopsy was positive for tumor, full doses of AMSA were given. A negative biopsy required a delay of chemotherapy until full hematologic recovery occurred (wbc count $\geq 3000/\mu l$; platelet count $\geq 100{,}000/\mu l$) and a 30-mg/m^2 reduction in dose.

RESULTS

Seventeen patients with SCCL refractory to conventional therapy were treated with AMSA, and 16 were evaluable for response. One patient was evaluable for toxicity only, because of early death from infection. Nine patients received one course, five received two courses, two received three courses, and one received four courses. One patient received three courses of AMSA at an escalated dose of 140 mg/m^2 with no increase in toxicity.

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No patient showed any regression of a measurable lesion after treatment with AMSA. Eight patients had rapidly progressive disease after the first AMSA course. Calculated from the first day of treatment, eight patients had initial disease stabilization (8–12 weeks) and then disease progression, and one patient had disease stabilization for > 16 weeks.

Toxicity data are based on the 19 fully evaluable AMSA courses in 13 patients. All patients whose wbc and platelet counts were not determined at a minimum of twice per week during the study were not evaluable for hematologic toxicity. The major side effect of AMSA therapy was leukopenia. In six of the 13 evaluable patients, the wbc count nadir was < 1000/μl, in four it was < 2000/μl, and in three it was < 3000/μl. In all of the evaluable courses the median wbc count was 1300/μl (range, 300–2900), and the median number of days to recovery was 20 (range, 14–30). One patient had a platelet count nadir of 12,000/μl and five patients had a platelet count of < 50,000/μl. The median platelet count in these six patients was 37,000/μl, with a median time to nadir of 10 days (range, 2–23). One drug-related death occurred from bacteremia secondary to leukopenia on Day 13 of AMSA therapy. Nonhematologic toxicity was mild and consisted of nausea (40%), vomiting (31%), and chemical phlebitis (6%). There was no evidence of cardiac, hematologic, renal, or hepatic toxicity. No evidence of any cumulative hematologic or nonhematologic toxicity was observed.

DISCUSSION

A schedule for AMSA of 120 mg/m² every 21 days was chosen in this phase II study on the basis of the known pharmacokinetics (8), as well as on dosing experience in a previous phase II clinical trial of AMSA in patients with renal cell carcinoma completed at this institution (9). Of the 16 SCCL patients who were treated with AMSA and were evaluable for response, eight (55%) had disease progression after the first course of chemotherapy; no complete or partial responses were observed. AMSA doses of 120 mg/m² every 21 days produced significant hematologic toxicity in refractory SCCL patients who had received intensive chemotherapy. Severe hematologic toxicity, coupled with the 55% immediate disease progression rate, leads us to believe that more frequent treatment would not be more beneficial.

AMSA at the dose and schedule used in this study has very limited antitumor activity in previously treated patients with SCCL. Although this does not preclude the possibility that AMSA may be active earlier in the course of therapy, the rapid disease progression and the lack of any objective evidence of response suggest that AMSA has very limited, if any, activity in SCCL.

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Ethinyl Estradiol and Medroxyprogesterone Treatment in Advanced Breast Cancer: A Pilot Study[1]

Brief Reports and Preliminary Communications

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SUMMARY

Twenty patients with disseminated breast cancer unresponsive to conventional chemotherapy and chemohormonotherapy were treated with an alternating sequential schedule of ethinyl estradiol and medroxyprogesterone on the basis of correlations between hormones and estrogen and progestin receptors. Of 19 evaluable patients, six underwent partial or complete remission, while five others showed minor responses.

[Cancer Treat Rep 65:135–136, 1981]

Additive hormonal treatment provides an effective means of controlling advanced breast cancer. Estrogens, androgens, antiestrogens, and high-dose progestins alone or in combination with polychemotherapy are the most currently used hormones (1–4).

Since 1974 our treatment strategy has included high doses of medroxyprogesterone acetate (MPA) followed, at relapse, by polychemotherapy. However, in an attempt to better control cases of very aggressive disease, we have used hormonotherapy and chemotherapy in a combined modality treatment (1). More recently we decided to treat patients unresponsive to conventional treatments with an alternating sequence of ethinyl estradiol (ETE) and MPA. This pilot study was essentially based on two considerations. First, some early papers reported a small beneficial response in about 20% of cases in clinical trials of estrogens in combination with progestins in advanced breast cancer (5–7). This approach was suggested by studies carried out on DMBA-induced tumors in rats that were then treated with estradiol-17 and progesterone (8). Second, the recent developments in our knowledge of hormone receptors and their interactions have shown that in human endometrial and breast cells, progesterone receptors are estrogen-dependent by an estrogen "priming" effect (3). Therefore, we considered the possibility that a short priming with estrogens, paving the cell with the progestin receptors, could improve the response to MPA treatment of hormone-dependent breast cancer cells.

MATERIALS AND METHODS

Twenty patients with advanced breast cancer were treated with the following sequential schedule: ETE, 1 mg orally on Days 1 and 2, then MPA (Farlutal-Farmitalia S.p.A.), 600 mg orally on Days 3–9. The cycle was repeated after a 2-day interval. All subjects were poor-risk patients who had already failed with hormonotherapy, chemotherapy, and hormonochemotherapy; moreover, all patients had

TABLE 1.—Characteristics of the 20 patients

Characteristic	No. of patients
Total evaluable	19
Median age: 57.5 yrs	
Menopause	20
Spontaneous	12
Ovariectomy	8
Sites of disease	
Soft tissue	18
Visceral tissue	5
Osseous tissue	10
Mixed	12
Prior treatment	20
Radical mastectomy	16
Radiotherapy	9
Chemohormonotherapy	20

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TABLE 2.—Results of treatment in 19 evaluable patients

Response	% reduction of initial lesions	No. of patients (%)
Complete response		2 (10.5)
Partial response	⩾ 50	4 (21.0)
Minor response	20–49	5 (26.3)
Complete or partial response	⩾ 50	6 (31.5)
Complete, partial, or minor response	⩾ 20	11 (57.8)
Stable disease		2 (10.5)
Disease progression		6 (31.6)

been previously unresponsive to high-dose MPA alone. The characteristics of the patients are summarized in table 1 and the response was assessed following the evaluation criteria of the International Union Against Cancer (UICC) (9). The median followup was 3 months (range, 1–6) and 19 of 20 subjects were evaluable. One patient was eliminated from the study for protocol violation.

RESULTS

Therapeutic responses.—Two complete remissions were noted (10.5%), both in patients treated for 6 months and affected by bone and soft tissue lesions (table 2). Four partial remissions occurred (21.0%), two in patients with soft tissue lesions and two in patients with soft tissue and visceral lesions. Minor remissions were noted in five patients (26.3%), and two had stable disease (10.5%). Six patients had disease progression and were eliminated from the study; one died after the appearance of CNS metastasis.

The overall response, consisting of the complete and partial remissions, was 31.5%; with the addition of the minor responses, this value reached 57.8%. The median duration of complete and partial responses was 3 months (range, 1–6).

Toxicity.—Side effects were practically absent; only one patient had hyperbilirubinemia of cholestatic origin that regressed after treatment suspension for progressing disease. Jaundice may be attributable to the disease itself or, less probably, to the estrogen; however, we never observed hyperbilirubinemia with the high-dose MPA treatment.

COMMENT

From these preliminary results it seems that the alternated sequential administration of ETE and MPA may be useful in managing advanced cancer, with acceptable overall remission rate and toxicity, in patients who already failed with conventional treatments.

The rationale of the sequence is suggested by interactions among hormones and receptors and the priming activity of estrogens on progesterone receptors. Although very preliminary, and subject to review after long-term followup and on larger patient populations, these results are quite suggestive. Other workers should extend this trial. If our results are confirmed, further studies in receptor-selected populations at different time intervals and hormone doses may allow a more physiopathologic approach to the treatment of some hormone-dependent tumors.

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Combination Chemotherapy for Advanced Ovarian Cancer: A Prospective Randomized Trial Comparing Hexamethylmelamine and Cyclophosphamide to Doxorubicin and Cyclophosphamide[1,2]

Brief Reports and Preliminary Communications

Peter E. Schwartz,* Roberta Lawrence, and Martin Katz[3,4]

SUMMARY

Forty-three patients with advanced stage III and IV epithelial cancers of the ovary were entered in a prospective randomized study comparing doxorubicin and cyclophosphamide to hexamethylmelamine and cyclophosphamide chemotherapy. No statistical difference in the overall response rates or survival times was demonstrated. However, the combination of doxorubicin and cyclophosphamide was more effective in inducing a response in histologically poorly differentiated cancers than in well-differentiated and moderately differentiated cancers, and the therapeutic implications of this observation are discussed.

[Cancer Treat Rep 65:137–141, 1981]

Prospective randomized studies have demonstrated that doxorubicin, cyclophosphamide, and hexamethylmelamine, administered as single agents, are each active in the management of advanced epithelial ovarian cancer (1–9). Studies subsequently were reported indicating that the combinations of hexamethylmelamine and cyclophosphamide (H-C) and doxorubicin and cyclophosphamide (A-C) were more effective in the management of advanced ovarian cancer than single-agent therapy alone (1,2,9–12). In 1976, a prospective randomized study at the Yale University Medical Center and its affiliated hospitals was initiated which randomized patients to either A-C or H-C chemotherapy for the primary treatment of advanced epithelial cancers of the ovary. This paper will present the results of that study.

MATERIALS AND METHODS

Each of the patients in the study was treated surgically at the Yale University Medical Center or at one of its affiliated hospitals. Twenty patients were randomized to receive A-C chemotherapy and 23 patients were randomized to receive H-C chemotherapy.

To qualify for the study, all patients must have had an estimated lifespan of at least 10 weeks and stage III (FIGO [International Federation of Gynecology and Obstetrics]) epithelial cancer of the ovary with significant residual cancer (> 2 cm, maximum diameter) left behind at initial surgery, or stage IV cancer. Histologic grading was based on the overall pattern of differentiation using "well-differentiated," "moderately differentiated," and "poorly differentiated" classifications which are readily reproducible and have been used in recent reports from other institutions (13,14). Tumors of borderline malignant potential were excluded from this study.

Doxorubicin was given initially at a dose of 40 mg/m² iv every 28 days, hexamethylmelamine was administered at a dose of 160 mg/m² orally for the first 14 days of each 28-day cycle, and cyclophosphamide was administered at a dose of 250 mg/m² orally for the first 5 days of each 28-day cycle. Dose reductions were determined by the degree of bone marrow suppression. Those patients who completed 2 years of chemotherapy were to undergo second-look surgery. Each patient in this study has been followed for at least 12 months or until death.

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TABLE 1.—Distribution by stage, histologic type, grade, response, and treatment

	No. of patients (%)	
	A-C (20 patients)	H-C (23 patients)
Stage (FIGO)		
III	15 (75.0)	18 (78.3)
IV	5 (25.0)	5 (21.7)
Histologic type		
Serous	15 (75.0)	17 (73.9)
Mucinous	1 (5.0)	3 (13.0)
Endometrioid	1 (5.0)	1 (4.4)
Other	3 (15.0)	2 (8.7)
Histologic grade		
Well-differentiated	5 (25.0)	6 (26.1)
Moderately differentiated	3 (15.0)	7 (30.4)
Poorly differentiated	11 (55.0)	10 (43.5)
Unknown	1* (5.0)	0 (0.0)
Response (evaluable patients)	17	20
Complete	6 (35.3)	6 (30.0)
Partial	4 (23.5)	4 (20.0)
No change	4 (23.5)	5 (25.0)
Disease progression	3 (17.7)	5 (25.0)

*1 patient did not have the primary cancer biopsied.

Estimation of survival probabilities and of median survival times were calculated using the life-table method (15). Gehan's modification of the Wilcoxon test (16) was used to compare survival curves. This test compares the complete survival experience of one group with the complete survival experience of a second group.

A chi-square test statistic for determining differences in rates and proportions (17) was used for testing the significance of the differences in the response rates, and the Fisher's exact test (18) provided the means of determining the results for those tables in which the frequencies were too small to be analyzed by the regular chi-square methods. The majority of patients in both treatment groups presented with stage III serous tumors (table 1).

RESPONSE

Patients were evaluated with regard to their most favorable response within the 12 months after the initiation of chemotherapy. A complete response was defined as the total disappearance of all palpable or measurable cancer for at least 3 months. A partial response was defined as a $\geq$ 50% reduction in the products of the diameters of the tumors, with effusions under control for a period of 3 months. No change was defined as a $<$ 50% reduction in the diameters of the measurable tumors for 3 months. Disease progression was defined as a $>$ 25% increase in the products of the diameters of any palpable or measurable cancer. No large differences between the two treatments in the distribution of response criteria were found. Three patients in each treatment group who had known cancer left behind at the initial surgery, but no clinically detectable cancer, were considered to be unevaluable for response.

Distribution by patient age, number of treatments, time from diagnosis to response, and duration of response were similar in the two treatment groups, and no statistical significance with regard to these variables was found. For the total sample, the mean age at diagnosis was 56.0 years (range, 38–70), the mean number of treatments was 8.3 (range, one to 24), the mean time from diagnosis to response was 2.2 months (range, 1–4), and the mean duration of response was 8.3 months (range, 3–30).

Survival curves for both treatment groups are compared in figure 1. The median survival from diagnosis was estimated at 16.7 months for the A-C-treated patients and 13.7 months for the H-C-treated patients. When tested, the survival experience of the A-C group was not significantly different from that of the H-C group.

A breakdown by histologic grade indicated that the A-C combination was particularly active in those patients who had poorly differentiated cancer (table 2). No patients with well-differentiated or moderately differentiated cancers responded to A-C therapy. This was statistically significant when com-

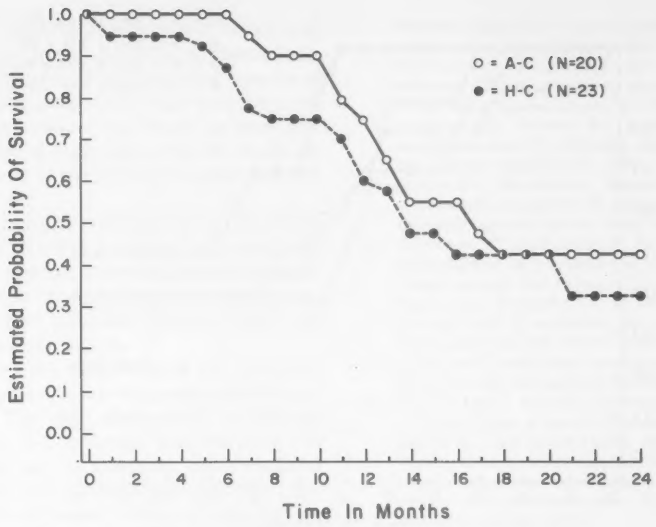

FIGURE 1.—Estimated probability of survival for the entire series of advanced ovarian cancer.

pared to the response to H-C therapy in the latter grades (0.50) ($P = 0.05$). The direction changed in the poorly differentiated category where the response rate for the A-C group was 0.90 and that for the H-C group was 0.50. Although the small sample sizes and the method of randomization must both be considered, the significant difference in response rate between the poorly and the more highly differentiated tumors within the A-C group (90% versus 0, respectively) should be noted (Fisher's exact test, $P < 0.001$). The estimated survival time was better for patients with poorly differentiated cancers treated with A-C than for those with better differentiated lesions, as shown in figure 2. The median survival time of the former was 18 months compared with a 12-month median survival time of the latter. The survival curves in the H-C groups are close.

TABLE 2.—Response to treatment by grade*

	No. of patients		
	A-C	H-C	Total
	Well-differentiated and moderately differentiated combined		
Response	0	6	6
No response	6	6	12
Total	6	12	18
Response rate	0.0	0.50	$P = 0.05$†
	Poorly differentiated		
Response	9	4	13
No response	1	4	5
Total	10	8	18
Response rate	0.90	0.50	$P = 0.09$†

*Complete or partial response. 3 patients in each treatment group were not evaluable for response; 1 patient in the A-C group did not have primary cancer biopsied.

†Fisher's exact test.

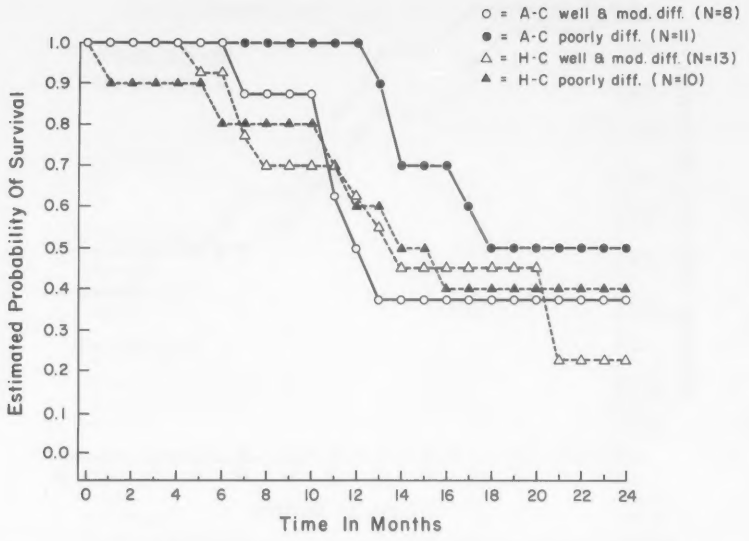

FIGURE 2.—Estimated probability of survival by histologic grade. 1 patient treated with A-C did not have the primary cancer biopsied.

TOXICITY

Severe hematologic toxicity (total wbc count < 1000/mm³) was only observed in four patients treated with A-C, the worst occurring in a patient with a total wbc count of 150/mm³; no severe thrombocytopenia (platelet count < 25,000/mm³) was observed in either treatment group.

To reduce the severity of nausea and vomiting, four of 20 patients treated with A-C chemotherapy and only one of 23 patients treated with H-C chemotherapy were changed to receive iv cyclophosphamide. This change to iv cyclophosphamide occurred late in most patients' treatment programs, at a time when objective responses were already established. It was therefore not considered appropriate to analyze this change as a separate prognostic factor. Two patients receiving A-C demonstrated cardiotoxicity with mild reversible congestive heart failure. Two patients developed significant complications with doxorubicin extravasation. The difference between the complication rate for A-C, 40% (eight of 20 patients), and that for H-C, 4.3% (one of 23 patients), was statistically significant ($P = 0.01$).

DISCUSSION

The introduction of combination chemotherapy for the treatment of patients with advanced epithelial ovarian cancers has resulted in increased response rates and survival from time of diagnosis (1,11,19). Prospective randomized studies are necessary to establish the role of the various combinations to determine which agents are most effective. There were no significant differences in survival rates when A-C and H-C patients are compared. However, in the group treated with A-C chemotherapy, the response to treatment was significantly greater and the survival time was considerably better among patients who presented with poorly differentiated tumors than among those with better differentiated ones. Survival with H-C chemotherapy was not very different between patients with well-differentiated and patients with poorly differentiated lesions.

The objective response rate of 58.8% (ten of 17 patients) for the A-C combination in the present study is consistent with recently published reports employing this therapy (2,9–12), and the response rate of 50% (ten of 20 patients) for the H-C combination is consistent with the 50% response rate with this combination reported from the M. D. Anderson Hospital and Tumor Institute (1).

The potential for utilizing tumor differentiation in selecting chemotherapy for advanced ovarian epithelial cancer has not received sufficient attention. Ozols et al (14) recently reported that histologic

grade may be an important prognostic factor and should be considered in the selection of chemotherapy. However, the latter study included tumors of borderline malignant potential which may present as advanced ovarian cancer and have an excellent prognosis (20). Such a diagnosis must be made on histologic assessment of the primary cancer and not its metastases (21).

In contrast to the report of Ozols et al (19), Ehrlich et al (22) reported a 56% complete response rate in undifferentiated ovarian epithelial cancers treated with PAC (cisplatin, doxorubicin, and cyclophosphamide) versus a 22% complete response rate with all grades of serous carcinoma.

In our study the only difference in the two prospective randomized arms was doxorubicin or hexamethylmelamine. The only statistically significant result was that the A-C program was not active in well-differentiated and moderately differentiated tumor whereas the H-C combination showed responses in all grades of tumor. Although the sample size is small, the question must be raised as to whether doxorubicin in combination chemotherapy is specifically effective in those poorly differentiated tumors which may have more actively dividing cancer cells.

We agree with Young (23) that grading in epithelial ovarian cancers may have significant therapeutic implications. It would seem important to review the grade of tumors previously reported in prospective randomized studies to see if the association with response to doxorubicin combination therapy is consistent. We believe that further studies might stratify for grade as well as volume of residual tumor for advanced epithelial cancers.

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Four-Drug Combination Chemotherapy in Extensive Non-Small Cell Lung Cancer: A Southwest Oncology Group Pilot Study[1,2]

Brief Reports and Preliminary Communications

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Results of combination chemotherapy for extensive non-small cell carcinoma have been disappointing, generally no better than single-agent chemotherapy or supportive care alone. The study described here was designed on the basis of (*a*) the improved survival reported for regimens containing doxorubicin and cyclophosphamide (1–5), (*b*) the single-drug efficacy of mitomycin C (6), and (*c*) the report by Japanese investigators of synergism for bleomycin with mitomycin C in the treatment of advanced cervical carcinomas (7). Other factors in the rationale were the optimal sequence of doxorubicin and cyclophosphamide (doxorubicin first) in an animal model system (8) and the therapeutic synergism of alkylating agent combinations in a broad spectrum of murine tumors (9), which we hoped might be applicable to cyclophosphamide and mitomycin C.

MATERIALS AND METHODS

Forty-five patients with extensive disease as defined by the criteria of the Veterans Administration Lung Study Group (10) were treated. All patients had documented histologic proof of lung cancer. Only six were women. The median age was 58. Distribution by cell type was adenocarcinoma in 16 patients, large cell anaplastic in 14, squamous cell in 14, and alveolar cell in one. Pretreatment performance status was fully ambulatory (Zubrod scale, 0–1) in only 14 patients, while 31 were less than fully ambulatory (in bed a significant portion of waking hours and incapable of normal activities; Zubrod scale, 2–4). None of the patients had received prior chemotherapy. All patients gave their written informed consent to treatment.

The chemotherapy schedule is shown in table 1; it consisted of doxorubicin, bleomycin, cyclophosphamide, and mitomycin C given in a sequence over 5 days and repeated at monthly intervals. This was done on an outpatient basis unless the patient's condition demanded hospitalization. Chemotherapy was repeated until obvious evidence of disease progression or unacceptable toxicity occurred. Complete remission (CR) required the complete disappearance of all clinically detectable evidence of tumor. Partial remission (PR) required a reduction of $\geq 50\%$ in measured tumor area or regression in evaluable but nonmeasurable tumor agreed upon by at least two independent observers (11). Lesser degrees of objective response, or responses with durations < 1 month, were classified as improvement. Initially, it was our intention to administer "consolidation" radiation therapy to areas of known prior involvement, plus elective whole-brain irradiation, in responding patients, based on considerations of patterns of relapse in a prior group-wide study (12). This was not done in an evaluable fashion due to the small number of responders.

RESULTS

Overall results are summarized in table 2. There were two CRs and three PRs in the entire group (11%). All but one of these patients had measurable disease. All but five of the 45 patients had measurable disease. If patients dying before 2 weeks in the study (3) are excluded as inevaluable, the response rate becomes 12%. Of the 14 fully ambulatory patients, three (21%) had responses, including one CR. Seven of the 14 fully ambulatory patients achieved response or "improved" status. There was no difference in response frequency by histologic type. The median duration of response was 17 weeks (range, 10–36).

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TABLE 1.—Chemotherapy schedule: 4-wk cycle

Drug	Dose	Schedule (days)	Route
Doxorubicin	40 mg/m^2	1	iv
Bleomycin	5 units	2,3,4	iv or im
Cyclophosphamide	600 mg/m^2	5	iv
Mitomycin C	5 mg/m^2	5	iv

TABLE 2.—Tumor response and patient survival

Group	No. of patients	No. of patients with—			Median survival (wks)
		CR	PR	Improvement	
Fully ambulatory	14	1	2	4	22
Less than fully ambulatory	31	1	1	3	9

Median survival was 13 weeks for the entire patient population, 22 weeks for fully ambulatory patients, and 9 weeks for less than fully ambulatory patients. The median survival of responders was 31 weeks versus 12 weeks for nonresponders.

The therapy was well-tolerated in general; although most patients had nausea, only seven had severe or prolonged vomiting and none required hospitalization as a result. Two of the 45 patients (4%) had drug-related deaths. One of these patients died from infection related to leukopenia, and one 62-year-old patient with severe pre-existing obstructive lung disease developed severe pulmonary fibrosis after two courses, which probably hastened his death. Leukopenia was observed in 90% of courses, with a mean granulocyte count nadir of 1100/mm^3; thrombocytopenia occurred in only 23%, with a mean platelet count nadir of 111,000/mm^3. However, three of five patients who received more than four courses and who had evaluable platelet count nadirs demonstrated thrombocytopenia at or after the fourth course, suggesting a cumulative effect probably related to mitomycin C. No stomatitis or dermatitis was observed.

DISCUSSION

The results of this combination of drugs offer no evidence of superiority over simpler regimens and may be inferior. The overall survival is probably not better than can be achieved with supportive care and no specific therapy (13). There appears to be no justification for further exploration of this particular drug regimen in extensive non-small cell lung cancer, although some of its individual components might bear further evaluation.

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Tolerance to Single-Dose Dactinomycin in Combination Chemotherapy for Solid Tumors[1]

Brief Reports and Preliminary Communications

Julie Blatt,* Michael E. Trigg, Philip A. Pizzo, and Daniel Glaubiger[2]

SUMMARY

We have reviewed our experience using single-dose dactinomycin (Act D). Twenty-nine patients with Ewing's sarcoma or rhabdomyosarcoma received 114 courses of Act D (2 mg/m²) in combination with vincristine, cyclophosphamide, or DTIC with or without radiotherapy. Side effects included nausea and vomiting (100% of the courses), severe thrombocytopenia (16%), granulocytopenia (18%), and mucositis (25%). In addition, skin reactions were observed in 74% of the courses following concomitant radiotherapy. These results were compared with those following 152 courses of combination chemotherapy which contained doxorubicin in place of Act D and which were administered to these same patients. It is concluded that single-bolus Act D is no more toxic, is at least as effective, and is more conveniently administered than the traditional divided daily dose of Act D.

[Cancer Treat Rep 65:145–147, 1981]

Dactinomycin (Act D) has proven important in the treatment of several pediatric neoplasms (eg, Ewing's sarcoma and rhabdomyosarcoma) (1–17), generally in combination with other agents (eg, cyclophosphamide and vincristine). In most regimens using Act D, a 5-day divided-dose schedule has been used (1–4). Recently, data have suggested that a single-dose schedule is equally effective and is well-tolerated (5). Since a single-dose schedule has the added advantage of requiring fewer hospital visits, the Pediatric Oncology Branch of the National Cancer Institute incorporated single-dose Act D into several of its solid tumor protocols. We report our experience with the toxicity of Act D using this dose schedule.

MATERIALS AND METHODS

The medical records of 29 patients who received single-dose Act D and for whom there were sufficient data to evaluate toxicity were reviewed. Nineteen patients had Ewing's sarcoma and ten had rhabdomyosarcoma. The patients ranged in age from 4 to 43 years (mean, 17).

Act D was administered iv at a dose of 2 mg/m² (no upper limit) in combination with vincristine, cyclophosphamide, or DTIC. Therapy was administered in 3–6-week cycles, with the exact interval determined by the time to hematopoietic recovery (ie, granulocyte count > 1500/mm³, platelet count > 100,000/mm³).

A total of 114 courses of combination chemotherapy containing Act D were given to these 29 patients. In 95 of the courses, Act D was given at full dose (2 mg/m²). In 19 courses, the dose was reduced by one half because of toxicity (*see* below). Twenty-three of the 114 courses containing Act D were administered either concomitant with or within 1 month following radiotherapy (RT) (1000 R/week × 5) to known disease sites. For comparison, 152 courses of combination chemotherapy which included doxorubicin (Adr) (35 mg/m²) in place of the Act D and which were administered to these same patients were also evaluated. Twenty-nine of the 152 Adr-containing courses were administered concomitant with RT.

The following measures of hematologic toxicity were evaluated: *thrombocytopenia*—total platelet count ≤ 20,000/mm³ (severe) or 20,000–100,000/mm³ (moderate); *anemia*—hemoglobin ≤ 8.0 g/100 ml; and *granulocytopenia*—absolute granulocyte count ≤ 500/mm³.

Nonhematologic toxicity was evaluated as follows: *gastrointestinal*—mucositis defined as subjective soreness with visible oral ulceration, nausea

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TABLE 1.—Toxicity following combination chemotherapy with and without Act D

Toxic effect	Act D, full dose, no RT (72 courses)	Act D, reduced dose (19 courses)	Act D + RT (23 courses)	No Act D* (152 courses)
Nausea and/or vomiting	72	19	22	140
Diarrhea	6	3	8	5
Mucositis	15	1	13	8
Fever, neutropenia	5	3	13	11
Thrombocytopenia				
< 20,000 cells/mm^3	7	4	8	4
20,000–100,000 cells/mm^3	6	3	4	4
Anemia	1	3	4	4
Acne	13	2	13	0
Radiation-enhanced erythema, cystitis, diarrhea	1	2	19†	0
Hepatitis	3	0	2	0

*Identical chemotherapy courses with Adr substituted for Act D.
†These 19 episodes involved only 3 patients.

and vomiting, diarrhea, or serum transaminase elevations > 100 IU unrelated to infection, tumor, or transfusions; *dermatitis*—acneiform rash or brisk erythema in an irradiated area, with or without moist desquamation; and *other toxic effects*—hemorrhagic cystitis or diarrhea due to radiation enteritis.

RESULTS

The toxic effects encountered with chemotherapy courses with and without single-dose Act D are summarized in table 1. Hematologic toxicity occurred in 61 of 114 (53%) chemotherapy courses containing Act D, compared with 23 of 152 (15%) chemotherapy cycles with Adr in place of Act D. The hematologic toxicity included severe thrombocytopenia (platelet count ≤ 20,000/mm^3) in 19 of 114 (16%) and granulocytopenia (polymorphonuclear neutrophils ≤ 500/mm^3) in 21 of 114 (18%) of the courses containing Act D. Concomitant RT, however, appeared to be responsible for much of this toxicity. Hematologic toxicity resulted in a delay in administration of subsequent chemotherapy in 15 of 114 (13%) courses. These delays were in no case believed to be responsible for tumor regrowth. Hematologic toxic effects in patients who received combination therapy with Adr in place of Act D included severe thrombocytopenia in four (3%) and granulocytopenia in 11 (7%) of 152 courses.

Erythema with moist desquamation of the skin in an irradiated area occurred in 17 of 23 (74%) courses following Act D and RT. These skin reactions were occasionally so severe that RT was interrupted. Acneiform rashes were also frequent in patients receiving Act D (28 of 114 courses; 25%), particularly in adolescents. Iv extravasation was not noted during any course of therapy. No radiation enhancement or recall of skin reactions could be attributed to Adr.

Mucositis was also more frequent with Act D (29 of 114 courses; 25%), particularly when it was given with RT (13 of 23; 57%). Nausea and vomiting was a common problem, regardless of the drugs administered. In no instance was drug toxicity related to mortality.

DISCUSSION

Act D has traditionally been given in doses of 0.10–0.15 mg/kg/day (upper limit, 0.5 mg/dose) for 5 consecutive days (1–4,7,8,16,17). With this regimen, patients generally experienced nausea and vomiting, diarrhea, mild hematologic toxic effects, and occasional stomatitis (1–4). The frequency of these side effects was similar to that described in our patients and that in other previously reported patients who received high single doses of Act D. For example, Benjamin et al (5) reported a phase I–II trial with single high-dose Act D in 33 patients. All patients had vomiting, two had diarrhea, and none had skin reactions. The lowest wbc count observed in these patients was 2300/mm^3; the lowest platelet count was 124,000/mm^3. No infections or hemorrhagic complications were reported.

It has been assumed that if Act D were given in single large doses as part of combination chemotherapy, it would be more toxic than when it is given by the intermittent schedule. However, the toxic effects observed in our patients with the high single-dose schedule were similar in incidence and often less severe than those reported when Act D was administered according to a divided-dose schedule as a part of combination chemotherapy (15–17).

Green (11) used Act D in a schedule similar to ours in combination with vincristine and cyclophosphamide and reported that of 31 patients, all had nausea and vomiting but only a "low incidence" of bone marrow suppression and stomatitis. However, among the eight patients with soft tissue sarcoma and the seven with Ewing's sarcoma, there were only two therapeutic responses.

Pharmacologic studies have suggested an increased therapeutic effect when Act D is given as a single bolus rather than as intermittent doses. Tattersall et al (14) demonstrated a 36-hour half-life in man when the drug is given as a 2-mg/m^2 iv push, and peak tumor tissue levels in mice have been found to be higher when the drug is given in this manner (5).

It appears that when Act D is used as a single dose of 2 mg/m^2 in combination chemotherapy, it is no more toxic than when it is used in an intermittent daily dose schedule. While schedules using Act D have greater hematologic toxicity than those using Adr instead, the toxicity is tolerable and has not been associated with unexpected morbidity. Moreover, our treatment results for patients with Ewing's sarcoma (12) and rhabdomyosarcoma (unpublished observations) using this single-dose schedule appear at this time to be at least as good as results obtained with divided Act D doses, when compared by stage and/or site of primary disease (16,17). Furthermore, single-bolus Act D has the additional practical advantage of requiring less hospital time for the patient. This schedule therefore constitutes a reasonable alternative to the divided-dose schedule.

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Cyclophosphamide, Hexamethylmelamine, Doxorubicin, and Cisplatin (CHAD) as Second-Line Chemotherapy for Ovarian Adenocarcinoma[1]

Brief Reports and Preliminary Communications

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SUMMARY

Twenty women with recurrent ovarian adenocarcinoma received a monthly four-drug combination of cyclophosphamide, hexamethylmelamine, doxorubicin, and cisplatin as second-line chemotherapy. There were no objective responses to this regimen. This is in contrast to the 49% response rate reported by Kane et al using these four drugs and the 63% response rate reported by Vogl et al using three of these drugs as second-line chemotherapy. The differences in the three regimens are reviewed; however, we could not identify reasons sufficient to account for the disparity in response rates.

[Cancer Treat Rep 65:149–151, 1981]

Using a monthly four-drug regimen of cyclophosphamide, hexamethylmelamine, doxorubicin, and cisplatin (CHAP), Kane et al (1) reported a 49% objective response rate in 35 women with advanced ovarian adenocarcinoma who had previously failed alkylating agent therapy. Seven (20%) of these women achieved a complete response. Using this same four-drug combination as primary therapy in advanced ovarian adenocarcinoma, Vogl et al (2) achieved a 90.4% response rate. These latter authors utilized three of these drugs, hexamethylmelamine, doxorubicin, and cisplatin (HAD), and achieved a 63% response rate in 22 women whose malignancy had progressed after alkylating agent therapy (3).

This report will detail our experience using the four-drug combination of cyclophosphamide, hexamethylmelamine, doxorubicin, and cisplatin (CHAD) in 20 women with recurrent ovarian adenocarcinoma whose malignancy had progressed after first-line chemotherapy. In addition, our results and regimen will be compared to CHAP and HAD as described by Kane et al (1) and Vogl et al (2), respectively. From June 1978 to December 1979, 21 women were entered in the protocol. One patient was not evaluable having been found at postmortem examination to have primary liver carcinoma and not ovarian carcinoma.

MATERIALS AND METHODS

All 20 patients had FIGO (International Federation of Gynecology and Obstetrics) stage III (16 patients with abdominal involvement) or IV (four patients) ovarian adenocarcinomas. Metastatic sites in the latter four patients consisted of lung metastasis in two, abdominal wall metastasis in one, and left supraclavicular lymph node metastasis in one. Histopathology consisted of serous adenocarcinoma in 11 patients, poorly differentiated adenocarcinoma in four, clear cell adenocarcinoma in three, and mucinous adenocarcinoma in two. The tumor grade was well-differentiated in three patients, moderately differentiated in four, and poorly differentiated in 13.

All 20 patients had failed with combination chemotherapy including high-dose methotrexate plus cyclophosphamide in 18 women and cyclophosphamide plus cisplatin in two. They had received one to 23 monthly courses of chemotherapy, for a total of 211 courses (median, nine), prior to receiving second-line chemotherapy. Five of the 20 women had objective responses (three complete and two partial) to the first-line chemotherapy. Two patients had had previous radiation therapy, one of the two with ip chromic phosphate. All 20 patients had measurable disease prior to initiating second-line chemotherapy. The median age was 50 years (range,

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TABLE 1.—Treatment

Drug	Days	CHAD	CHAP	HAD
		Dose and method of administration		
Cisplatin	1	1 mg/kg 5% dextrose in 0.45 normal saline Furosemide Mannitol 6 hrs	50 mg/m^2 5% dextrose in 0.45 normal saline Furosemide Mannitol 1 hr	50 mg/m^2 5% dextrose in 0.45 normal saline Furosemide Mannitol Bolus
Doxorubicin	1	25 mg/m^2	40 mg/m^2	30 mg/m^2
Cyclophosphamide	1	400 mg/m^2	300 mg/m^2	
Hexamethylmelamine	1–14		150 mg/m^2	
	8–21	150 mg/m^2		200 mg/m^2
Pyridoxine	8–21	300 mg		
		No. of patients/response rate		
		20/0%	35/49%	22/63%

21–80). Performance status[3] was 0 in seven patients, 1 in nine, 2 in one, 3 in three, and 4 in none.

Table 1 shows the doses and method of administration of the CHAD, CHAP, and HAD regimens. Patients received from one to 19 courses of CHAD (median, three), for a total of 82 courses. Prior to each course of chemotherapy all patients had a physical examination, creatinine clearance, SMA-12 including BUN and creatinine, cbc, chest roentgenogram, and ECG.

Eligibility requirements prior to entry in this study consisted of a wbc count > 3000/mm^3, a platelet count > 100,000/mm^3, normal liver function as measured by SMA-12, and normal renal function as measured by SMA-12 and creatinine clearance. CHAD was repeated every 4 weeks if the wbc count was > 3000/mm^3, and if the platelet count was > 100,000/mm^3, with treatment being delayed 1 week if these values were not obtained. All patients received the CHAD regimen at a minimum of every 4 weeks and a maximum of every 6 weeks. The CHAD regimen was continued until the patient exhibited progression of disease.

A complete response was defined as the complete disappearance of all measurable disease lasting for a minimum of 1 month. A partial response was defined as a ≥ 50% reduction in the product of the diameters of the measurable tumor lasting ≥ 1 month. Stable disease was defined as the absence of growth in measurable disease. Disease progression was defined as a ≥ 25% increase in measurable disease and/or the appearance of a new lesion.

[3]Performance status: 0 = asymptomatic; 1 = ambulatory with symptoms; 2 = < 50% bedridden; 3 = > 50% bedridden; and 4 = 100% bedridden.

RESULTS

Toxicity

There were no treatment-related deaths. Nausea and vomiting was severe (> 300 ml/day) in nine of the 20 patients. No patient developed hearing loss or neurologic, renal, hepatic, or cardiotoxic effects. One patient developed ECG changes, ie, minimal decrease in the size of the QRS complexes. Severe leukopenia (< 1000 cells/mm^3) and severe thrombocytopenia (< 25,000 cells/mm^3) occurred in two patients, each necessitating dose reduction. The remaining 18 patients had platelet count nadirs of 78,000–186,000/mm^3 and wbc count nadirs of 1500–3000/mm^3.

Response and Survival

None of the 20 patients receiving CHAD had an objective response. Fourteen patients had disease progression: seven after one or two courses of CHAD and seven after the disease had stabilized for ≥ 3 months. Six women continue to receive CHAD chemotherapy; all had stable disease for ≥ 3 months. The median survival is > 4 months (range, 3–20) for those with stable disease and 3 months (range, 3–8) for those with progression of disease.

DISCUSSION

We used CHAD to treat 20 patients whose disease had progressed after first-line chemotherapy. None of the patients had an objective response. This is in contrast to the 49% objective response rate of

Kane et al using these same four drugs in patients who had failed treatment with alkylating agents. Moreover, Vogl et al, using three of these drugs as second-line chemotherapy, achieved a 63% objective response rate.

Although there are differences in the three regimens, the reasons for the contrasting results between CHAD and CHAP or HAD are not readily apparent. The minor differences between the CHAP and CHAD regimens are the slightly higher doses of cisplatin and doxorubicin used in the CHAP regimen. The major differences, however, are the longer period of infusion of cisplatin in our regimen (6 hours vs 1 hour) and the fact that we administered hexamethylmelamine on Days 8–21 as compared to Days 1–14 in Kane's CHAP regimen. The longer cisplatin infusion was chosen because prolonged infusions have been associated with prolonged elevations in serum.[4] The major differences in the HAD combination of Vogl et al as compared to our CHAD combination are the lack of cyclophosphamide and the fact that cisplatin was given by bolus in the HAD regimen. The slightly increased doses in the CHAP and HAD regimens are offset by the fact that 90% and 45% of the patients had significant dose reductions, respectively, as compared to 10% of our patients. Because some of the neurologic toxicity of hexamethylmelamine resembles that of pyridoxal phosphate deficiency, all of our patients also received pyridoxine (4). This was apparently not administered in the CHAP or HAD regimens.

Finally, the CHAP and HAD regimens may have been effective as second-line chemotherapy because the patients' disease had progressed primarily after alkylating agent chemotherapy. This is in contrast to all 20 of our patients whose disease had progressed after combination chemotherapy. The latter situation may preclude the use of the CHAP, HAD, or CHAD regimen.

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Kinetic and Cytotoxic Effects of Cytarabine and Daunorubicin on Cells in Culture[1,2,3]

Brief Reports and Preliminary Communications

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SUMMARY

To investigate the possibility that a significant kinetic basis exists for the improved clinical response often obtained using drug combinations, Chinese hamster ovary cells were exposed to cytarabine and daunorubicin in combination. Cell survival was determined by a colony-counting assay; kinetic perturbations were monitored by flow cytometry. No major synergistic effects of the combination were seen; in fact, under certain conditions, each drug appeared to provide some protection against the lethal effects of the other. By pairing a moderately toxic concentration of one drug with a nontoxic concentration of the other, we observed sequence-dependent differences in survival. These differences could not be attributed simply to alteration of the cell cycle phase positions by the nontoxic drug, combined with phase-specific lethality of the toxic drug. It is likely, however, that daunorubicin provided partial protection from cytarabine-related cytotoxicity through its effect on the DNA synthesis rate.

[Cancer Treat Rep 65:153–156, 1981]

The dramatic improvement in the clinical responses of patients with leukemia and other malignant neoplasms to combination chemotherapy as compared with earlier single-drug treatment has provided the impetus for investigations of possible mechanisms of synergistic or antagonistic drug effects. In many cases, however, it is not clear whether the improved clinical response is due to synergistic drug interaction against the tumor cells themselves, or rather to a relatively decreased toxicity of the combination to the normal host cells. Moreover, the role of drug-induced cell cycle perturbation in the observed response has not been adequately explored.

In the present study, we employed a model system of cells in culture to ascertain whether the combination of daunorubicin (DNR) and cytarabine (ara-C) resulted in synergistic lethality. Although this study utilized asynchronous cells, the cell line selected, Chinese hamster ovary cells, can be readily synchronized by relatively nonperturbing methods (1,2) and can maintain good synchrony for comparatively long periods. This cell line would therefore be well-suited to further detailed investigation of cell cycle dependencies of drug action or interaction.

DNR and ara-C were selected for investigation because of their therapeutic synergism for induction therapy in acute nonlymphocytic leukemia (3,4). Edelstein et al (5) found that ara-C followed by DNR resulted in synergistic lethality of leukemic colony-forming units in mice but had only additive effects against normal colony-forming units. It is not known, however, whether the distributions of cells among the phases of the cell cycle were significantly altered by this treatment, and if so, whether the observed effects on survival were directly related to differential effects on cell progression.

In the combined drug studies reported here, our objectives were to determine (*a*) whether the use of a relatively nontoxic dose of one drug, followed by (or preceded by) a moderately toxic dose of the other, would result in either enhanced or decreased cytotoxicity; and (*b*) whether sequence-dependent differences in the cell cycle phase distributions could be correlated with differences in survival. In these experiments, the drugs were added either simul-

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[3]Presented in part at the Third Annual Meeting of the Cell Kinetics Society, Miami, FL, Mar 15–17, 1979.

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taneously or separated by drug-free intervals of either 1 or 4 hrs. In order to determine the cell cycle phase distributions at the time of exposure to the more toxic drug in each case, samples were obtained for flow cytometric measurement of DNA content.

MATERIALS AND METHODS

Chinese hamster ovary cells (CHO-Kl) were obtained from the American Type Culture Collection, Rockville, MD, as CCL 61. They were grown in plastic tissue culture flasks (Falcon) in Ham's F12 medium without antibiotics and supplemented with 10% fetal calf serum purchased from Grand Island Biological Co, NY. Cells were incubated in a humidified 37°C incubator in an atmosphere of 5% CO_2. They were subcultured twice weekly and had a doubling time of ≈ 12 hrs.

Ara-C was obtained from the Upjohn Co, Kalamazoo, MI, while DNR was obtained as daunorubicin hydrochloride from the Division of Cancer Treatment, National Cancer Institute, Bethesda, MD. The drugs were diluted in 0.9% NaCl. Exponentially growing cells were detached with 0.05% trypsin, washed, and replated in 5.0 ml medium in 60-mm petri dishes. One hour later, when most cells had reattached, 0.2 ml of drug-containing saline was added. After incubating the cells for the desired time in the presence of drug, some plates were stained for flow cytometry, while others were rinsed three times with fresh medium and reincubated. When cells were to be exposed to two drugs sequentially, the second drug was added at the appropriate time and subsequently washed out as before. Four days later the medium was changed, and after a total of 6 days of incubation, colonies were stained with crystal violet and counted for determination of survival.

Distributions of cells through the cell cycle were obtained by flow cytometry, using propidium iodide as the fluorochrome (6). Cellular fluorescence was measured on a Cytofluorograf model 4802 (Ortho Instruments, Inc, Westwood, MA), and data analysis was performed using a PDP 11/70 computer as previously described (7,8). Preliminary experiments established that neither drug affected the fluorescence distributions at the concentrations used here.

RESULTS AND DISCUSSION

In the two sets of experiments presented here, exponentially growing cells were exposed for 1 hr to ara-C and DNR, either simultaneously or sequentially. In each case, the higher dose of one drug was paired with the lower dose of the other. Drug concentrations were selected such that the more toxic drug, when administered alone, was lethal to about one-half the population, while the less toxic drug was selected to be the highest concentration tested that was essentially nonlethal. In both cases, the less toxic drug caused cells to accumulate in S and/or G_2/M phase after 6 and 24 hrs exposure (not shown).

Ara-C (10^{-6} M) + DNR (10^{-7} M)

In a series of dose-survival experiments to select appropriate drug levels for the present study, we found that virtually all cells survived a 1-hr exposure to ara-C at 10^{-6} M, while about 70% survived a similar exposure at 10^{-5} M. Sixty percent of the cells survived a 1-hr exposure to DNR at 10^{-7} M. After exposure of the cells to ara-C at 10^{-6} M and DNR at 10^{-7} M, either simultaneously or separated by 1- or 4-hr drug-free intervals, survival rates were as shown in table 1. Also listed in the table are the distributions of cells among the phases of the cell cycle (including subphases of S) immediately prior to exposure to the more toxic drug (DNR).

Since the expected survival, under the assumption that the drugs acted independently, was 59%, it is clear that the drugs did not induce synergistic lethality under these conditions. On the contrary, there appeared to be some drug interference when the drugs were added simultaneously (72% survival). It would be relevant to know whether the observed differences in survival were related primarily to alterations in cell cycle phase positions induced by the nontoxic drug before exposure to the more toxic agent. This cannot be the case for the 1-hr drug-free interval, since when ara-C was given first, the cell cycle distribution (including subphases within S) immediately prior to exposure to DNR (A-1-D in table 1) was nearly the same as when cells were not perturbed before DNR exposure (D-1-A and D + A). Thus, because the cell cycle phase distributions at the time of exposure to DNR were nearly the same regardless of the order of drug exposure, the observed differences in survival cannot be attributed to differences in cell cycle phase positions.

Additional evidence that lethality is not necessarily correlated with cell cycle position is seen for the 4-hr intervals between drug exposures. Survival rates for the DNR-first regimen versus adding the two drugs simultaneously were significantly different (58% versus 72%) despite the fact that their cell cycle phase distributions were the same (table 1).

TABLE 1.—Cell cycle phase distributions and survival rates of cells exposed to ara-C (10^{-6} M) and DNR (10^{-7} M)

Drug*	% cell survival†	% of cells in phase—‡								
		G_1	S	G_2/M	S_1	S_2	S_3	S_4	S_5	S_6
A-1-D	53 ± 6	24	65	11	23	7	8	8	7	12
D-1-A	64 ± 4									
D+A	72 ± 3	28	61	11	28	5	6	5	6	11
D-4-A	58 ± 3									
A-4-D	62 ± 1	21	66	13	21	6	10	10	8	11

*1st letter indicates the drug added first (A = ara-C, D = DNR); the No. = the drug-free interval between exposures to the 2 drugs (1 or 4 hrs); D+A indicates that the 2 drugs were given simultaneously.

†Each value = the mean of 3 samples ± 1 SD. Survival rates after exposure to the individual drugs alone were: ara-C, 100%; DNR, 59%. According to Student's t test at the 5% level of significance (ref 10), *underlined values* = survival rates that were significantly different from expected if the drugs had acted independently.

‡Values = flow cytometry-derived % of cells in the phases and subphases of the cell cycle immediately before exposure to the more toxic drug (DNR in this case). When DNR was added first (or together with ara-C), this distribution was the same as the control.

TABLE 2.—Cell cycle phase distributions and survival rates of cells exposed to ara-C (10^{-4} M) and DNR (0.5×10^{-7} M)*

Drug	% cell survival†	% of cells in phase—								
		G_1	S	G_2/M	S_1	S_2	S_3	S_4	S_5	S_6
D-1-A	39 ± 2	26	60	14	20	6	8	7	7	12
A-1-D	27 ± 2									
A+D	24 ± 1	28	61	11	28	5	6	5	6	11
A-4-D	23 ± 3									
D-4-A	32 ± 2	24	54	22	25	4	6	5	6	8

*Refer to table 1 for definitions.

†Survival rates after exposure to the individual drugs were: ara-C, 32%; DNR, 93%. Each value = the mean of 3 samples ± 1 SD. *Underlined values* = survival rates that were significantly different, at the 5% level of significance, from expected if the drugs had acted independently.

Ara-C (10^{-4} M) + DNR (0.5×10^{-7} M)

In these experiments, ara-C was the more toxic drug (32% survival at 10^{-4} M when given alone for 1 hr), while DNR was virtually nontoxic at 0.5×10^{-7} M (93% survival). As seen in table 2, when cells were exposed to DNR first, with a 1-hr interval between drug exposures, the survival was significantly greater than when ara-C was given first (or simultaneously with DNR). However, the cell cycle phase distributions at the time of exposure of cells to ara-C were nearly the same. The differences in survival, therefore, cannot be attributed to DNR-induced alteration in cell cycle phase positions.

For the 4-hr time interval between drugs, exposure of the cells to DNR first resulted in their accumulation in the G_2 phase (row D-4-A of table 2). Although the survival for this sequence was close to the expected value (30%), it was significantly higher than for the drugs given together (or ara-C first). Thus, when DNR was given first, it seemed to reverse the modest synergistic effect of the combination. This apparent reversal may have been due simply to the DNR-induced accumulation of cells in the ara-C-resistant G_2 phase.

In summary, we have found that, while drug-induced changes in the distribution of cells through the cycle may have been responsible for the increased survival in one case, this mechanism was not the cause of sequence-related survival differences in the other examples. The data do not exclude the possibility that alteration in the *rate* of cell progression (as contrasted with alteration in position of the cells within the cycle) by the nontoxic drug altered the response of the cells to the more toxic drug. This is especially likely to have occurred when ara-C was the more toxic agent. Since ara-C is highly S-phase-specific, it is plausible that a DNR-induced decrease in the rate of DNA synthesis (9) could have provided partial protection against ara-C-induced lethality.

That such protection was not evident when ara-C and DNR were added simultaneously might have been because the cytotoxic effect of ara-C became fixed before the protective effect of DNR became significant. To the extent that the drug response of this system resembles that of leukemic or other tumor cells in vivo, the results suggest that therapeutic synergism achieved by administering ara-C and DNR sequentially is due to factors other than cell cycle redistribution induced by the drugs.

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Uptake of Melphalan by Human Ovarian Carcinoma Cells and Its Relationship to the Amino Acid Content of Ascitic Fluid[1]

Brief Reports and Preliminary Communications

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SUMMARY

Melphalan uptake by human ovarian carcinoma cells obtained from patients with clinically documented disease was significantly reduced by ascitic fluid. Examination of the ascitic fluid revealed physiologic concentrations of a number of amino acids, notably leucine (100–150 μM) and glutamine (500–600 μM). These two amino acids, when used at concentrations found in the ascitic fluid, significantly reduced melphalan uptake to levels which approximated those obtained with ascitic fluid. The possible effect of high concentrations of these amino acids on melphalan therapy for this neoplasm is discussed with particular reference to the model melphalan transport system in the murine L1210 leukemia cell. Specific emphasis is placed on the rationale for combining melphalan with arginine and a glutamine-depleting enzyme.

[Cancer Treat Rep 65:157–161, 1981]

Melphalan was originally synthesized in England (1,2) and Russia (3) with the hope that attachment of the amino acid phenylalanine to the nitrogen mustard group would enhance its antitumor activity. The drug has a broad range of antitumor activity in animals (4) and against human neoplasia where it is used in the treatment of multiple myeloma (5,6), ovarian carcinoma (7,8), breast cancer (9), and malignant melanoma (10). Such antitumor activity has been shown to be reduced by certain competitive amino acids (11). This reduction in cytotoxicity is primarily due to the L-isomers of leucine and glutamine (11) and has been correlated with reduced cellular uptake of the drug (12,13). As a result of these observations, the present study was undertaken to determine whether ascitic fluid from patients with clinically documented ovarian carcinoma contains leucine and glutamine in sufficient concentration to alter the uptake of the drug.

MATERIALS

Sulfosalicylic acid (ACS certified reagent grade) and lithium hydroxide were purchased from Fisher Scientific Co, Silver Spring, MD. Bovine serum albumin was obtained as serum fraction V from Miles Laboratories, Inc, Elkhart, IN. Dulbecco's phosphate-buffered saline was supplied by the National Institutes of Health media unit. Leucine and glutamine were obtained from Calbiochem, San Diego, CA. The silicone oil, Versilube F-50 (specific gravity, 1.045 at 25°C; viscosity, 70 centistokes at 25°C), was obtained from Harwick Chemical Corp, Cambridge, MA. Melphalan (11 millicuries/mmol), labeled in the chloroethyl groups with ^{14}C, was synthesized by Mr. Morris Leaffer under contract with Stanford Research Institute, Menlo Park, CA. Melphalan solutions were prepared fresh in 75% ethyl alcohol containing an equimolar concentration of hydrochloric acid. Further dilutions were made in aqueous medium immediately prior to use in order to minimize hydrolysis.

METHODS

Preparation of Ascitic Fluid for Amino Acid Analysis

Peritoneal fluid was removed by paracentesis and immediately placed on ice. The pH of the fluid was

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7.4. All subsequent processing of the fluid was performed at 4°C. The fluid was centrifuged at 300 × *g* for 10 minutes to remove cells and was then processed by a standard cell fractionation scheme to remove any cell organelles present in the ascitic fluid. The 300 × *g* supernatant fraction was centrifuged at 800 × *g* for 10 minutes to remove nuclei and then at 11,000 × *g* for 15 minutes to yield a postmitochondrial supernatant which was centrifuged for 60 minutes at 100,000 × *g* to pellet ribosomal material. Six milliliters of the postribosomal supernatant was then deproteinized by the addition of 4 ml of 10% (wt/vol) sulfosalicylic acid. The precipitate was removed by centrifugation at 11,000 × *g* for 15 minutes and the supernatant was adjusted to pH 2.6–2.8 with lithium hydroxide, filtered through 0.22-μ filters, and frozen at –20°C. Amino acid analysis was performed on a Beckman 121 M amino acid analyzer using lithium citrate buffers (pH 2.83, 0.2 N; pH 3.70, 0.2 N; pH 3.75, 1.0 N).

Melphalan Uptake Studies

Ascitic fluid containing human ovarian carcinoma cells was centrifuged at 60 × *g* for 5 minutes to remove rbcs. The resulting cell suspension, which contained approximately 90% ovarian cells, was either resuspended in cell-free peritoneal fluid or in Dulbecco's phosphate-buffered saline containing 0.1 mM bovine serum albumin and 15 mM glucose (PAG). The cells suspended in PAG were washed two additional times in the same buffer system to remove any extracellular amino acids. [^{14}C]-melphalan was then added to yield a drug concentration of 1.5 μM to cells in PAG or ascitic fluid or with 150 μM L-leucine or 500 μM L-glutamine to cells in PAG. This drug dose was chosen because it approximates the concentration of melphalan achievable in the plasma 1–3 hours following oral melphalan treatment (14). Aliquots (600 μl) of the respective cell suspension were placed on a 1.0-ml cushion of silicone oil and the cells were pelleted at the indicated time points by centrifugation for 1 minute at 12,000 × *g*. Tips containing the cell pellets were removed and the pellets were solubilized in 0.2 N NaOH for 3 hours at 37°C, neutralized with acetic acid, and counted on a Beckman liquid scintillation counter.

TABLE 1.—Amino acid concentrations in the ascitic fluid of 2 patients with ovarian carcinoma

Amino acid	Amino acid concentration (μM)	
	Patient 1	Patient 2
Phosphoserine	19.7	16.0
Taurine	125.7	122.7
Phosphoethanolamine	None detected	None detected
Aspartic acid	None detected	None detected
Threonine	134.4	167.0
Serine	119.0	148.0
Asparagine	20.3	13.3
Glutamic acid	65.5	247.0
Glutamine	512.0	620.0
Proline	140.0	147.0
Glycine	439.1	455.0
Alanine	807.2	390.7
Citrulline	129.5	64.7
α-Amino-*n*-butyric acid	145.3	119.3
Valine	277.8	182.5
Cystine	94.7	109.8
Methionine	27.3	24.6
Cystathionine	7.0	2.4
Isoleucine	125.7	58.5
Leucine	149.8	111.8
Tyrosine	55.6	55.3
Phenylalanine	51.8	55.5
Ethanolamine	39.2	49.0
Ornithine	43.3	23.8
Lysine	142.7	137.1
1-Methyl histidine	27.7	None detected
Histidine	60.0	48.8
3-Methyl histidine	8.9	2.9
Arginine	52.4	28.6

Protein Determination

Protein was determined by the method of Bradford (15).

CASE REPORTS

Patient 1

Patient 1 was a 34-year-old white female who had a 1-month history of abdominal swelling. On July 23, 1978, the patient had an exploratory laparotomy and a 7-cm right ovarian tumor was removed. Pathologically, the tumor was a poorly differentiated papillary adenocarcinoma of the ovary. At surgery, extensive intra-abdominal disease with ascites was also noted. She was referred to the National Cancer Institute (NCI), Bethesda, MD, for further treatment of her malignancy. At the time of her initial evaluation at the NCI she was noted to have pleural effusions and mediastinal adenopathy. On August 13, 1978, she started combination chemotherapy with cyclophosphamide, hexamethylmelamine, 5-FU, and cisplatin. On September 27, 1978, because of increasing ascites and discomfort, a paracentesis was performed. Cytology was consistent with ovarian cancer. Routine acid fast bacilli and fungal cultures were negative and blood cell counts on the fluid revealed an rbc count of 500/mm^3 and a wbc count of 50/mm^3 (differential count on 100 cells showed one polymorphonuclear leukocyte, 25 lymphocytes, 73 histiocytes, and one mesothelial cell). Total protein on the fluid was 3.9 g/100 ml (serum, 5.4). Immediately after removal, the peritoneal fluid was prepared for amino acid analysis as described above.

Patient 2

Patient 2 was a 51-year-old white female who had a 6-week history of lower abdominal pain. A tumor was noted on pelvic examination. Exploratory laparotomy on September 26, 1978, revealed a large tumor which pathologically was a clear cell adenocarcinoma of the ovary. There were multiple implants of tumor throughout the abdominal cavity. She had a debulking procedure and was referred to the NCI for further treatment of her stage III ovarian cancer. At the time of her initial evaluation at the NCI she was noted to have ascites. A paracentesis was performed on October 31, 1978. Cytology on the fluid was positive for tumor consistent with ovarian cancer. Routine acid fast bacilli and fungal cultures were negative and blood cell counts on the fluid revealed an rbc count of 2600/mm^3 and a wbc count of 600/mm^3 (differential count on 100 cells showed 21 polymorphonuclear leukocytes, 25 lymphocytes, 39 histiocytes, and 15 abnormal cells). Total protein on the fluid was 3.9 g/100 ml (serum, 5.1). Immediately after removal, the peritoneal fluid was prepared for amino acid analysis as described above.

RESULTS AND DISCUSSION

The ascitic fluid of both patients with clinically documented ovarian carcinoma contained physiologic concentrations of several amino acids (table 1). The concentration of leucine and glutamine was considered high enough to warrant consideration of these two amino acids as potential inhibitors of melphalan uptake by human ovarian carcinoma cells. It had been previously shown that melphalan cytotoxicity to L1210 murine leukemia cells was reduced by these amino acids (11). Melphalan uptake studies with the same tumor cell indicated that the reduced cytotoxicity was due to a reduction in drug uptake (12,13). As shown in figure 1, melphalan uptake is considerably reduced by incubation of human ovarian cancer cells with drug in ascitic fluid, an observation which can be attributed to the high concentration of leucine and glutamine (table 1). Leucine and glutamine, at concentrations present in the ascitic fluid, reduced melphalan uptake by human ovarian carcinoma cells (fig 1).

During the course of work with the murine L1210 leukemia cell system, several important relationships between melphalan transport and cytotoxicity evolved. Foremost, melphalan cytotoxicity ranges from minimal to complete within a small critical range of net drug uptake. Thus, small interferences in drug uptake result in large decreases in cytotoxicity (12). This has been shown to be due to a reduction in the concentrative uptake of melphalan by natural carrier substrates. Leucine completely abolished concentrative uptake (16) due to its higher affinity for the transport carrier (12). The results described in the present study suggest that concentrative uptake of pharmacologically achievable concentrations of melphalan is significantly reduced by amino acids in the ascitic fluid and may contribute to the development of resistance to melphalan by exposure of cells to suboptimal levels of drug.

The ability of physiologic concentrations of leucine (100–200 μM) and glutamine (400–600 μM) to reduce concentrative uptake of melphalan (12) and thereby decrease drug cytotoxicity (11) suggests that removal of these amino acids may result in an improved therapeutic response to melphalan. This is supported by studies with the murine L1210 leukemia cell (17) which demonstrated that nearly all of the reduced cytotoxicity of melphalan found in

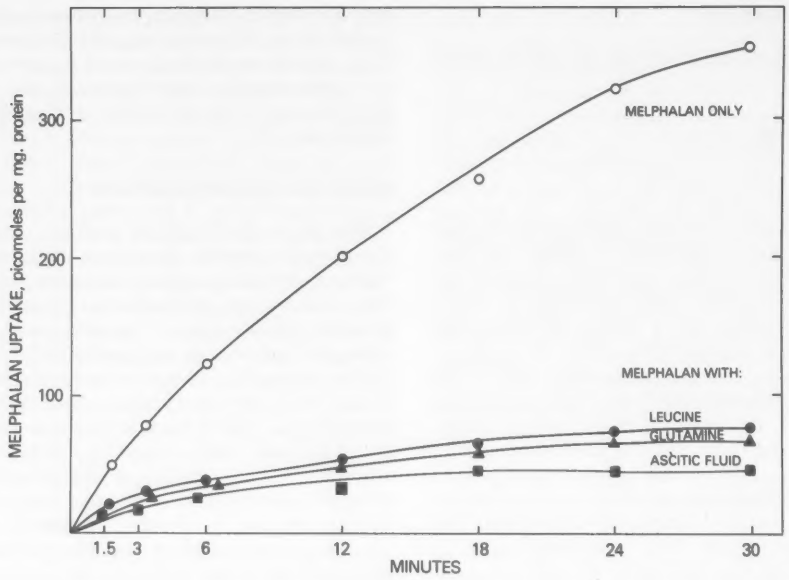

FIGURE 1.—Reduction in melphalan uptake by human ovarian carcinoma cells by ascitic fluid, leucine, and glutamine. Human ovarian carcinoma cells, prepared as described in the Methods section, were incubated with 1.5 μM ^{14}C-melphalan in PAG (○), in the same medium with 150 μM L-leucine (●), in the same medium with 500 μM L-glutamine (▲), or in ascitic fluid (■). 600-μl aliquots were removed in triplicate and centrifuged at 12,000 × *g* for 1 min at the indicated time points. Tips containing the cell pellets were processed as described in the Methods section. Ovarian carcinoma cells were obtained from patient 1.

an amino acid environment can be attributed to leucine and glutamine.

A potential strategy to increase the cytotoxic potential of melphalan in an amino acid environment containing glutamine and leucine would be to first deplete glutamine by ip administration of glutaminase:asparaginase and then to administer melphalan with arginine or α-amino-γ-guanidinobutyric acid via the same route to reduce the therapeutic interference provided by leucine. Glutamine-depleting enzymes from *Acinetobacter glutaminasificans* and *Pseudomonas aureofaciens* (18) have been isolated. Studies in this laboratory (13) demonstrated that depletion of glutamine with *Acinetobacter* glutaminase:asparaginase enhances melphalan uptake in vitro and results in a decrease in the surviving fraction of cells. Importantly, these studies also demonstrated that the products of glutamine deamination, glutamic acid and ammonia, do not interfere with melphalan transport or cytotoxicity. The ability of α-amino-γ-guanidinobutyric acid to increase intracellular drug and resulting cytotoxicity in the presence of leucine was maximal when leucine and α-amino-γ-guanidinobutyric acid were present in equimolar concentrations (17). The molar ratio of arginine to leucine in the ascitic fluid of both patients is considerably < 1 (table 1) and suggests that the therapeutic potency of melphalan might be increased by concomitant administration of arginine or α-amino-γ-guanidinobutyric acid with melphalan. Alternatively, the use of peritoneal lavage to remove amino acids prior to ip drug administration would appear to be worthy of consideration.

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Phase II Study of Cyclophosphamide and Cytarabine in the Treatment of Metastatic Adenocarcinoma of the Colon and Rectum

A review of tumor cell growth kinetics shows that growth fraction and subsequent sensitivity to chemotherapeutic agents, especially antimetabolites, is inversely related to tumor cell mass (1). These data constitute one rationale for the use of chemotherapy as an adjuvant treatment for micrometastatic disease after removal of the primary tumor (bulk of cell mass) by surgery or radiation therapy. Cell cycle non-specific chemotherapeutic agents can be used in a manner similar to surgery to decrease tumor cell mass and increase growth fraction. Studies in a hamster plasmacytoma model by Schabel have shown that animals treated with cyclophosphamide had a significant percent increase in lifespan, but all died of tumor despite manipulation of dose and schedule. This model tumor was not significantly sensitive to cytarabine as a single agent, but treatment with cyclophosphamide followed by cytarabine resulted in a 53% cure rate (2). The cell cycle non-specific agent cyclophosphamide decreased cell mass and increased growth fraction, allowing significant effect of a cycle-specific agent, cytarabine (1,2).

Support for the use of cyclophosphamide and cytarabine in gastrointestinal cancer is derived from studies of the mouse colon model 26 which shows synergistic effect for a sequential combination of cyclophosphamide and cytarabine.[1] Based on these concepts, the present study of the combination of cyclophosphamide and cytarabine was undertaken in refractory metastatic colorectal cancer.

METHODS

Fourteen patients (mean age, 56 years) with histologically proven, advanced colorectal cancer were entered into the trial. The duration of metastatic disease prior to cyclophosphamide and cytarabine therapy was 9 months (range, 0–20). All patients had at least one measurable lesion, and all had previously been treated with 5-FU and methyl-CCNU and other cytotoxic drugs (table 1).

No patient had received chemotherapy or radiation therapy within 4 weeks.

[1]Schabel FM. Biology and treatment of murine colon carcinoma. Report to National Large Bowel Cancer Project. Southern Research Institute, Birmingham, AL, Sept 1976.

TABLE 1.—Characteristics of 14 patients entered in the study

Characteristic	No. of patients (%)
Primary	
Colon	5 (36)
Rectal	9 (64)
Metastatic involvement	
Liver	12 (86)
Lung	3 (22)
Mesentery omentum	4 (29)
Nodes	3 (22)
Bone	1 (7)
Prior chemotherapy*	
MC + F	14 (100)
MC + F + V	8 (57)
MC + F + V + M	5 (36)

*MC = methyl-CCNU; F = 5-FU; V = vincristine; M = mitomycin C.

Pretreatment evaluation included complete history and physical examination, tumor measurements, performance status, cbc and platelet count, urinalysis, battery of serum chemistries and enzymes, CEA, electrocardiogram, chest roentgenogram, liver scan, bone scan, and bone roentgenograms of scan-positive areas. Cbc and platelet counts were done every 10 days, while tumor measurements, chest roentgenograms, CEA, and serum chemistries were repeated every 3 weeks. The definition of an objective response was standard. In all patients, the pretreatment wbc count was $> 4000/mm^3$, the platelet count was $> 100{,}000/mm^3$, and the Karnofsky performance status was $\geq 70\%$.

A model schedule for the administration of cyclophosphamide and cytarabine was provided by F. M. Schabel, using an L1210 mouse leukemia system. These data show optimal percent increase in lifespan, using cyclophosphamide on Day 1, followed by cytarabine on Day 2, every 3 hours × 8 (F. M. Schabel, personal communication). Using this model, the present protocol was designed to give the cyclophosphamide (800 mg/m^2) by rapid iv injection on Day 1, followed by cytarabine (200 mg/m^2) by 24-hour infusion beginning 24 hours after cyclophosphamide injection. Courses were repeated every 3 weeks for two cycles and were continued if measurable disease remained stable.

RESULTS

Of the 14 patients entered into the study, 12 were completely evaluable for response (minimum of two

cycles of therapy). One patient refused therapy after one cycle of treatment and died from progressive disease 7 weeks after initiation of the trial. A second patient died 2 weeks after the first cycle from rapidly progressive disease; an autopsy showed massive liver metastases and death from hepatorenal syndrome. There were no objective responses among the 12 completely evaluable patients. One patient did have stable disease with unchanged malignant hepatomegaly and stable CEA for 5 months. After seven cycles he developed ureteral obstruction secondary to retroperitoneal tumor mass and a tenfold increase in CEA.

Toxicity was primarily hematologic, moderate, and predictable. Five patients had nadir wbc counts of 1000–1999/mm^3 at 10 days, with one patient having a wbc count of 350/mm^3 that was associated with sepsis from which she recovered without sequelae. Two patients had nadir platelet counts of 50,000–100,000/mm^3, and one had a platelet count nadir of 23,000/mm^3 without bleeding. There was moderate nausea and vomiting in the majority of patients on Day 1 after cyclophosphamide administration, but this was controlled with antiemetics and lasted < 24 hours in all patients. No cystitis was observed.

One patient developed a significant and previously unreported toxic reaction consisting of fever and chills during cytarabine infusion and a subsequent diffuse maculopapular skin eruption. Skin biopsy showed a leukocytoclastic vasculitis with eosinophilic infiltration. The patient recovered with appropriate treatment. There are no previous reports of vasculitis with cytarabine, and this potentially severe untoward effect will be reported separately.

DISCUSSION

The need for more effective treatment of metastatic colorectal carcinoma has been widely discussed. Despite evidence of in vivo success of the combination of cyclophosphamide and cytarabine in several systems (2), including a mouse colon system with sensitivities paralleling human colon carcinoma,[2] we found no responses among 14 patients with colorectal cancer refractory to 5-FU and methyl-CCNU. This disparity may arise because human colon cancers are probably even less sensitive to cyclophosphamide (3) and cytarabine (4) as single agents than is Corbett's colon model 26. Alternatively, the proliferation kinetics of even the slowest growing solid tumor models may be sufficiently different from those of human colon cancer that predictions of cytoreduction, recruitment, and sensitivity to S-phase agents is unreliable. Lastly, the dose and schedule chosen were less toxic than anticipated and may have been suboptimal. However, this particular regimen was inactive in advanced colorectal carcinoma already refractory to combination chemotherapy.

[2]*See* footnote 1.

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Late Reinforcement of Maintenance Therapy in Children With Acute Lymphoblastic Leukemia

In spite of advances in management of acute lymphoblastic leukemia (ALL) in children (1–5), the first complete remission still ends in bone marrow relapse in a large proportion of patients. The responses to reinduction therapy are better in chil-

dren who relapse in bone marrow after cessation of therapy than in those who relapse during therapy (3). Relapse while patients are still receiving therapy carries a grave prognosis. The relapse rate for the first year after therapy was higher than that for the next 3 years (1).

It does seem that durable complete remission can be achieved in patients who relapse after a long period of elective cessation of therapy (4). So far we have stopped therapy in 46 children with ALL, and bone marrow relapses have occurred in eight patients. Two children died in relapse; six patients are in second remission, and in two of them therapy has been stopped again (5). We have now introduced the late reinforcement of maintenance therapy (according to Memphis protocol VII) to reduce the frequency of relapses or to delay them. In complete remissions lasting 2½ years, just before treatment is discontinued the following therapy is given: on Days 1–5 and 8–12, one daily dose of 100 mg/m² of cytarabine (ARA-C) iv or sc, and 8 hours later, one dose of 100 mg/m² of 6-thioguanine (TG) orally. The combination of ARA-C and TG is frequently used in the LSA_2-L_2 protocol for treatment of children with non-Hodgkin's lymphoma in the consolidation phase (6). We have treated 20 children with the non-Hodgkin's lymphoma LSA_2-L_2 protocol, and after two 5-day cycles of ARA-C and TG, myelotoxicity was frequently observed but did not prove to be a life-threatening complication.

We reason that, late in remission, patients with ALL should be better able to tolerate the ARA-C and TG combination: a regimen different from that previously used in maintenance therapy.

Late intensification maintenance therapy in acute myeloid leukemia has been undertaken and is considered to be promising (6–8).

The experience of other centers is necessary in order to determine whether late reinforcement of maintenance therapy will be useful.

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Zinostatin-Related Pulmonary Toxicity[1]

Zinostatin is an investigational antitumor drug currently undergoing phase II clinical trials. Side effects found in phase I clinical studies included anorexia, nausea, vomiting, diarrhea, leukopenia, thrombocytopenia, fever, skin rash, anaphylaxis, and hepatic and renal abnormalities (1,2). Other rare side effects were headache, stomatitis, fatigue, and chills. Recently, Seltzer et al (3) reported on a patient who developed an acute pulmonary reaction characterized clinically by dry cough associated with hypoxemia and pathologic changes in the lung similar to those found in bleomycin pulmonary toxicity.

In the phase I–II studies of 76 patients at the M. D. Anderson Hospital and Tumor Institute, we observed one patient who developed a pulmonary toxic reaction associated with zinostatin therapy.

CASE REPORT

The patient was a 62-year-old black male diagnosed in December 1976 as having well-differen-

[1]Supported in part by contract N01-CM-57042 from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

tiated lymphocytic lymphoma. He smoked approximately two to three packs of cigarettes per day for 40 years but had no significant history or physical signs of pulmonary disease. He was initially treated with chlorambucil and prednisone for 2 months. In February 1977, he was referred to our hospital. Physical examination revealed a well-nourished, well-developed male with enlarged cervical, axillary, and inguinal lymph nodes; hepatosplenomegaly; and a perianal mass. The heart and lungs were normal. Laboratory studies showed the following values: hemoglobin, 8.9 g/100 ml; hematocrit, 29%; wbc count, 278,000/mm^3 with 96% lymphocytes; and platelet count, 85,000/mm^3. SMA-12/60 was within normal limits except for an alkaline phosphatase of 109 milliunits/ml (normal range: 30–85) and an LDH of 243 milliunits/ml (normal range: 100–225). Bone marrow aspiration and biopsy revealed well-differentiated lymphocytic lymphoma. Chest roentgenogram showed hilar adenopathy and slight prominent lung markings. ECG was within normal limits.

On February 14, 1977, chemotherapy was initiated using cyclophosphamide, doxorubicin, and prednisone. The patient received a total of seven courses of this drug regimen at 3-week intervals.

On further progression of the disease, zinostatin (3600 units/m^2/day × 5 days iv at 4-week intervals) was started on August 4, 1977. After receiving three courses of zinostatin, the patient experienced shortness of breath and increasing cough but had no chest pain and was afebrile. There were crepitant rales at both lung bases and examination of the heart was unremarkable. Chest roentgenogram on October 14, 1977, showed bilateral pulmonary infiltrates and a left pleural effusion without any cardiomegaly. ECG showed occasional atrial ectopic beats but was otherwise unremarkable. The patient was initially treated with digoxin and diuretics but showed no response. Pleural fluid was transudate in nature and was negative for pathogens and malignant cells. Repeated sputum cultures showed normal throat flora. Toxoplasma and cytomegalic virus titers were normal. Arterial blood gas studies showed gradually increasing hypoxemia and respiratory alkalosis. Pulmonary function tests revealed severe obstructive and restrictive defects with poor mechanical lung function. Pulmonary ventilation scans showed normal distribution of radioactivity in both lungs. Because of increasing dyspnea with no obvious etiology, an open lung biopsy was done on November 3, 1977. The pathologic findings included diffuse interstitial fibrosis and lack of any inflammatory cells. There were interesting vascular abnormalities consisting of thickening and occlusion of pulmonary veins and hypertrophy of muscular pulmonary arteries. The stains for *Pneumocystis carinii* and fungi were unremarkable. Microbiologic cultures of the specimen were negative. During his hospitalization, the patient had progressively worsening dyspnea with slowly deteriorating arterial blood gases. On December 15, 1977, he had cardiopulmonary arrest and died.

COMMENT

The clinical findings and the time course of lung toxicity in our patient were quite similar to those reported by Seltzer et al (3). Both patients had received 2–4 months of zinostatin before pulmonary symptoms first appeared. Pulmonary toxic effects in our patient occurred while he was receiving a higher dose of zinostatin (3600 units/m^2/day × 5) compared to that received by the patient reported by Seltzer (2250 units/m^2/day × 5). The roentgenographic findings of interstitial pulmonary infiltrates were similar in both cases. In addition, our patient developed a unilateral pleural effusion. The histologic changes in these two cases were similar to those found in bleomycin pulmonary toxicity (4). In addition to quite marked interstitial fibrosis and septal thickening, there were vascular changes in the form of venous thrombi and obliteration, perhaps related to endothelial damage. The pulmonary reaction observed in our patient could theoretically be related to his prior exposure to cyclosphosphamide or chlorambucil; however, the temporal relationship of pulmonary symptoms to zinostatin therapy supports a cause and effect relationship. Moreover, pulmonary toxic reactions to alkylating agents typically occur after prolonged exposure (4).

Encouraging clinical data from Japan on the activity of zinostatin generated considerable interest and led to further evaluation of this agent in phase I–II studies in the US. However, the preliminary results of these studies have shown a disappointing lack of activity against both leukemia and solid tumors (5). Accordingly, the future prospects of zinostatin as a useful antitumor agent appear quite limited and, therefore, its pulmonary toxicity is of little clinical significance.

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Phase II Evaluation of PALA and AMSA for Patients With Disseminated Malignant Melanoma[1]

PALA, an inhibitor of aspartate transcarbamylase, was selected for clinical trials because of activity against the B16 melanoma model.[2] The agent was active on all schedules and substantially increased the survival of laboratory animals versus controls. Phase I studies have identified the dose-limiting toxic effects as dermatologic (rash) and gastrointestinal (diarrhea) (1). AMSA, an acridine derivative, has achieved increases in lifespan ranging from 11% to 205% in the B16 melanoma model (2). A phase I investigation identified myelosuppression as dose-limiting and recommended a monthly iv dose of 90–120 mg/m² (3).

By random selection, patients with biopsy-proven malignant melanoma were treated with PALA (22 patients) or AMSA (18 patients). There were 25 males and 15 females with a median age of 58 years. Eleven patients had had prior chemotherapy; 33 patients had Eastern Cooperative Oncology Group performance status of 0–1 and seven had a performance status of 2–3; 28 patients had visceral disease and 12 had soft-tissue nodal disease.

The doses were: PALA, 5.0 g/m²/hour each 3 weeks, and AMSA, 90–120 mg/m²/hour each 4 weeks. Response was defined as a ≥ 50% decrease in the product of the longest perpendicular diameters of the most clearly measurable indicator lesion without new lesions or significant symptomatic deterioration. Patients with evaluable disease were assessed by recently reported criteria (4).

Two of 16 patients receiving PALA as initial treatment had regressions (table 1). One patient had a > 50% decrease for 3 months by computerized tomography scan of a biopsy-proven maxillary sinus mass. Another patient achieved objective regression of measurable lung lesions for 7 months. One of 15 patients receiving PALA as subsequent treatment had a regression (axillary node, 1.5 months). There were no objective regressions among 13 patients receiving AMSA as primary treatment (table 1). One of 19 patients responded to AMSA as subsequent treatment (lung lesion, 5.7 months).

TABLE 1.—Results of treatment by agent used and site of metastasis*

Regimen	Visceral-dominant	Nonvisceral
Primary		
PALA	2/12	0/4
AMSA	0/7	0/6
Subsequent		
PALA	1/11	0/4
AMSA	1/15	0/4

*Values = No. of objective regressions/No. of evaluable patients.

Eight of 31 (26%) patients receiving PALA had moderate to severe nausea, vomiting, or diarrhea. Four patients developed a transient, diffusely erythematous truncal rash. Among AMSA recipients, four of 32 (12.5%) had moderate to severe nausea and vomiting. Sixteen patients had leukopenia ($< 4.1 \times 10^3$ wbcs/mm³) and three had thrombocytopenia ($< 130 \times 10^3$ platelets/mm³) without sequelae.

In the dose and schedule in our study, neither PALA nor AMSA displayed substantial activity against malignant melanoma.

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[2]Clinical brochure: PALA (NSC-224131). Prepared by the National Cancer Institute, Bethesda, MD, Dec 1977.

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Phase II Study of AMSA in Patients With Metastatic Malignant Melanoma[1]

AMSA is a lipid-soluble acridine derivative which has shown promising antitumor activity in murine models (1). In phase I trials AMSA has produced acceptable toxic effects, primarily myelosuppression (2–4).

Thirty-five patients with histologically confirmed metastatic malignant melanoma (objectively measurable disease, wbc count > 4000/mm³, platelet count > 150,000/mm³, and Karnofsky performance status [PS] ≥ 50) were entered into this study. AMSA was administered as single iv infusions every 3 weeks. The initial dose was 120 mg/m² for patients with PS ≥ 70 and 90 mg/m² for those with PS < 70. If no substantial myelosuppression was seen, subsequent doses were increased by increments of 30 mg/m² until myelosuppression or progression of disease occurred.

Patients were considered evaluable either after two courses of therapy or after obvious progression of disease with one course of treatment. Therapeutic responses have been defined previously (3). Thirty-four of 35 patients were evaluable for response to therapy with AMSA. Characteristics of adequately treated patients are listed in table 1.

Responses.—Two patients (6%) had partial responses to AMSA therapy. One previously untreated 23-year-old female had 80% regression of a pelvic mass; she continued in remission for 10 months. Another 34-year-old woman who had been previously treated with DTIC had 90% shrinkage of multiple subcutaneous nodules and complete disappearance of massive ascites for 9 months. However, in this second case, the patient had been treated with conjugated estrogens for 6 months after undergoing bilateral oophorectomy for metastasis to the ovary. Hormone treatment was stopped simultaneously as AMSA was started, and although tumor shrinkage may have been due to AMSA treatment, it is also possible that the tumor was hormonally dependent (5) and responded to estrogen withdrawal. Two additional patients had minor responses with AMSA treatment.

Toxicity.—Patients were treated with a median of two courses (range, one to 12). Three patients received a maximum dose of 90 mg/m², 23 received 120 mg/m², and eight received ≥ 150 mg/m². The major toxic effect was myelosuppression, particularly wbc depression (table 1). The wbc and platelet count nadirs generally occurred between Days 10 and 18 (median wbc count nadir, 3500/mm³ and median platelet count nadir, 220,000/mm³). One patient with bone and bone marrow metastases had life-threatening toxic reactions with a wbc count nadir of 100/mm³ which was successfully treated. No cumulative or persistent hematologic toxic effect was observed and no drug-related deaths occurred. Other toxic effects included mild nausea and vomiting in six patients (18%), headaches or dizziness in two, and mucositis in one. No hepatic, renal, or cardiac dysfunction was noted in association with AMSA therapy.

In summary, after treatment with AMSA, we observed only two partial and two minor responses in 34 patients with visceral dominant metastatic melanoma. One half of the patients, 17 of 34, had not received prior chemotherapy and only one of

TABLE 1.—Characteristics, toxicity, and responses of adequately treated patients

Characteristic	No. of patients
No. of patients	34
Age in yrs	
Median: 52	
Range: 21–69	
Sex	
Male	15
Female	19
PS	
Median: 80	
Range: 50–90	
Previous chemotherapy	
Yes	17
No	17
Sites of measurable disease	
Soft tissue	22
Lung	20
Liver	2
Abdominal/pelvic mass	2
Toxicity	
Wbc count nadir (cells/mm³)	
< 1000	1
1000–3999	18
≥ 4000	15
Platelet count nadir (cells/mm³)	
< 99,000	4
100,000–149,000	4
≥ 150,000	26
Response	
Partial	2
Minor	2

[1]Supported in part by Public Health Service grant CA-05826 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

these 17 previously untreated patients (6%) responded to AMSA therapy. With only two major responses (6%), we conclude that AMSA at this dose and schedule has a true major response rate < 15% ($P < 0.05$) and is only a marginally effective single agent in the treatment of visceral metastatic melanoma.

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Bleomycin, Vincristine, and Methotrexate (BOM) in Squamous Cell Carcinoma of the Lung: A Phase II Study

Between January 1977 and December 1978 the North Milan Cooperative Group admitted 31 patients with histologically confirmed stage III unresectable squamous cell carcinoma to a bleomycin, vincristine, and methotrexate (BOM) regimen. BOM was chosen on the basis of the increase in survival observed by Samuels et al (1) in association with radiotherapy. The schedule was: bleomycin, 45 mg continuous infusion on Days 2–4; vincristine, 2 mg iv on Day 1; and methotrexate, 25 mg iv on Days 5, 12, and 19 every 5 weeks. Conditions for eligibility included performance status (PS) > 4, no previous chemotherapy or radiotherapy, estimated survival > 3 months, wbc count ≥ 4000/mm³, platelet count ≥ 150,000/mm³, and presence of measurable lesions. Of 31 patients, 22 were evaluable, three were excluded for insufficient therapy, and six were lost to followup. None of the 22 patients attained an objective response; 16 (73%) had stable disease, ie, < 50% change in the initial size of the lesion for at least 3 months from the beginning of therapy. Only one patient with M_1 disease attained stabilization, and all but one of the patients with M_0 disease had stable disease. PS 8–9 was more frequent in patients with stable disease. The median duration of stabilization was 8.8 months, with four patients having stable disease 12 months from the beginning of the study. The median survival time (MST), calculated according to Kaplan and Meier (2), was 9.8 months, 14 patients with stable disease having an MST of 11.2 months and the four patients with disease progression having an MST ranging from 4 to 5.5 months (table 1). At 18 months, 30% of the patients with stable disease were still alive.

Toxicity was very low, with nausea, vomiting, and stomatitis occurring in only three patients. One patient had mild neurotoxic reactions; no pulmonary toxicity of bleomycin was seen.

Two conclusions can be drawn. First, BOM appears to be inactive but despite the lack of objective responses, BOM led to survival comparable to other regimens in non-small cell lung cancer (3). Second, there was little toxicity from BOM.

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TABLE 1.—Response and survival according to stage and PS

Response	No. of patients	MST		No. of patients with stage III of—		No. of patients with PS of—	
		Mos	No. of patients	M_0	M_1	6–7	8–9
Stable disease	16	11.2	14	15	1	2	14
Disease progression	6	4–5.5	4	1	5	2	4
Totals	22	9.8	18	16	6	4	18

tard) in squamous lung cancer. Experience in fifty patients. Cancer 37:1237–1242, 1976.

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[1]This study was done by the North Milan Cooperative Group at Cattedra di Patologia Speciale Medica V, Ospedale L. Sacco, Università degli Studi di Milano (*A. Libretti* and *E. Piazza*); Seconda Divisione di Medicina Generale, Ambulatorio Oncologico, Ospedale Fatebenefratelli-Fatebenesorelle (*A. Scanni, M. Tomirotti,* and *M. Biraghi*); Prima Divisione di Medicina Generale, Ospedale Civile, Vimercate (*R. Scapaticci, C. Bianchi,* and *G. Restelli*); Istituto Nazionale per la Riabilitazione e Cura dell'Anziano, Istituto di Ricerca, Casatenovo (*G. Scarpazza* and *M. P. Calzavara*); Istituto di Ricerche Farmacologiche "Mario Negri" (*S. Marsoni* and *L. Morasca*); Milan, Italy.

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Hexamethylmelamine, Doxorubicin, and Cyclophosphamide in Advanced Ovarian Cancer Resistant to Previous Chemotherapy

From February 1978 to January 1980, 31 patients with epithelial ovarian cancer (18–68 years old) who had not responded to or who had relapsed after previous chemotherapy were admitted to this study. They received hexamethylmelamine (HMM), 150 mg/m²/day orally on Days 1–14; doxorubicin, 50 mg/m² iv on Day 1; and cyclophosphamide (CP), 100 mg/day orally on Days 1–14 every 4 weeks (HAC). If wbc counts fell to < 3000/μl or platelet counts to < 100,000/μl, treatment was deferred until these values were restored.

Definitions were as follows: complete response (CR), the disappearance of all evidence of disease for at least 1 month; partial response (PR), ≥ 50% reduction in the sum of tumor diameters for at least 1 month; no change, < 50% reduction or < 50% disease progression for at least 1 month; and disease progression, > 50% increase in the sum of tumor diameters or the appearance of a new tumor mass.

Complete physical examinations and laboratory tests were made before each cycle. Peritoneoscopy and/or laparotomy was used to verify CRs and also to evaluate response when it was not clinically assessable (ie, diaphragmatic or peritoneal neoplastic lesions) or not exactly quantifiable by pelvic examination alone.

RESULTS

Table 1 shows the response with HAC in the 30 evaluable patients with ovarian cancer. One patient was not evaluable because she died before the end of the first treatment cycle. The median number of treatment cycles was three. The overall response rate (CR plus PR) was 16.7%, with CR in only one case (3.3%) lasting 10 months. PRs lasted 2, 4, 7, and 11 months. Median survival was 11 months (range, 4–24) for responders and 10 months (range, 3–26) for nonresponders. Four of the five responders had been treated before with alkylating agents only (one had also received 5000 rad on the abdomen), and one responder had also received doxorubicin (total, 150 mg/m²).

Five patients with only diaphragmatic or peritoneal lesions were apparently free of disease at pelvic examination, but peritoneoscopy showed that two had disease progression and three had no change.

All patients had nausea and alopecia. No life-threatening toxicity occurred after HAC therapy, and severe hematologic toxicity was infrequent. One patient, who had already received pelvic ra-

TABLE 1.—Response to HAC according to previous chemotherapy in 30 patients with ovarian cancer

Previous chemotherapy	Total No. of patients	No. of patients (%) with— CR	PR	No change	Disease progression
Single alkylating agent: CP or melphalan	25	—	4	8	13
CP + doxorubicin*	3	1	—	1	1
CP + methotrexate + 5-FU	2	—	—	1	1
Totals	30	1 (3.3)	4 (13)	10 (33.3)	15 (50)

*Total dose of doxorubicin did not exceed 150 mg/m².

diotherapy, developed severe, prolonged leukopenia (wbc count < 2000/μl; platelet count < 75,000/μl) after the second cycle of treatment, so the doses of all three drugs were halved.

DISCUSSION

The HAC combination showed minimal efficacy in ovarian cancer in this study. The overall response rate was ≈ 17% with only one CR (3%). Median survival was not improved.

There appears to be no other published data on this three-drug combination in alkylator-resistant ovarian cancer. The combination of CP, HMM, doxorubicin, and cisplatin in comparable patients reportedly produces response rates of 49% and 67% (1,2). Since the HMM and doxorubicin doses we used were at least as high as those in the above studies, presumably cisplatin was instrumental in the greater efficacy reported.

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Phase II Trial of Zinostatin in Children With Late-Stage Acute Lymphocytic Leukemia: A Pediatric Oncology Group Study[1]

Zinostatin has produced single-agent complete response rates of 40%–50% in Japanese trials in previously untreated adult patients with acute leukemia (1). A phase II trial was therefore initiated by the Pediatric Oncology Group (POG) to determine if zinostatin might be active in acute lymphocytic leukemia (ALL) in children.

Zinostatin was given at a dose of 6000 units/m²/day for 5 days by continuous infusion. After 21 days a second 5-day infusion was given at a dose of 7000 units/m²/day if the patient did not have progressive disease.

Patients were eligible for study if they (*a*) were < 18 years of age at the time of diagnosis and < 21 years of age at the time of relapse, (*b*) were in bone marrow relapse (M-3 marrow status), (*c*) were re-

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sistant to drugs of proven value in ALL, (*d*) had not received chemotherapeutic treatment for at least 1 week with the exception of prednisone, and (*e*) were not in a toxic condition from prior therapy.

Twenty-six patients were evaluable. The medians and ranges for the patient characteristics were: age at diagnosis, 5.7 years (0.4–15.2); number of previous remissions, three (zero to six); survival from diagnosis to study entry, 31 months (10–86).

Two patients attained a complete bone marrow remission after one course of therapy. None of the remaining patients demonstrated improvement in their bone marrow status. Zinostatin had a pronounced suppressive effect on normal and abnormal peripheral blood elements necessitating increased rbc and platelet transfusion support. Other toxic effects were nausea and vomiting (controlled with antiemetics) in seven patients, mild transient hepatotoxic effects in two patients, irreversible renal failure in one patient, and extravasation ulcer in one patient.

With an observed complete response rate of 7.7% (two of 26 patients), we can be 95% confident that the true response rate is $< 23\%$ (2). In the dose schedule employed, zinostatin can not be recommended as an induction agent in children with advanced ALL.

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ANNOUNCEMENTS

Health Information Management Program

Stephens College, Columbia, Missouri

Stephens College, Columbia, Missouri, has received unqualified 3-year accreditation for its Health Information Management Program. This is an external baccalaureate degree program for medical record administration offered through Stephens College Without Walls, which enables people 23 years of age and older to complete the bachelor of arts degree by independent study with Stephens faculty. Candidates must first complete a required entrance course scheduled throughout the year on campus and in several other locations in the country. The program allows job-bound accredited medical record technicians to progress to the level of registered record administrator without relinquishing job, family, social, or other community commitments.

For further information, contact: Eileen Marsh, Office of Public Information, Stephens College, Columbia, MO 65215 (314-442-2211, ext 338).

Abstracts from the European Society for Medical Oncology

Abstracts from the Sixth Annual Meeting of the European Society for Medical Oncology held in Nice, France, December 6–8, 1980, are now available from the Secretariat, F. Fein, Bibliothèque, Centre Antoine-Lacassagre 36, Voie Ronaire, 06054 Nice, Cedex, France.

Postgraduate Courses on Clinical Cancer Chemotherapy

Jakarta, Indonesia, May 11–16, 1981
Colombo, Sri Lanka, September 7–11, 1981

The International Union Against Cancer (UICC) is organizing two postgraduate courses on clinical cancer chemotherapy.

The first course, scheduled for May 11–16, 1981, in Jakarta, Indonesia, is being held at the invitation of the Kanker Lembaga Indonesia and the University of Indonesia in Jakarta. It will cover the major aspects of medical oncology, particularly patient workup and management. The international faculty for the course will include Drs. K. Brunner (Switzerland), Chairman; M. A. Friedman (US); J. Levi (Australia); M. Tattersall (Australia); and J. M. A. Whitehouse (United Kingdom).

The second course, scheduled for September 7–11, 1981, in Colombo, Sri Lanka, will be held in conjunction with the Fifth Asia-Facific Cancer Conference and at the request of the Sri Lanka Cancer Society. The Chairman of the International faculty will be Dr. J. M. A. Whitehouse (United Kingdom).

For further information, please contact: David W. Reed, Assistant to the Director, International Union Against Cancer, rue de Conseil-Général 3, 1205 Geneva, Switzerland.

First International Symposium on the Podophyllotoxins in Cancer Therapy

Southampton, England, July 8–9, 1981

The First International Symposium on the Podophyllotoxins in Cancer Therapy will be held July 8–9, 1981, at the Medical and Biological Sciences Building, University of Southampton, Southampton, England. The podophyllotoxin derivatives have been undergoing evaluation for over 10 years and appear to represent a new class of anticancer agent. The First International Symposium will review the work of the past decade and explore the current and future roles of these agents in cancer therapy. The program will be divided

into four sessions and will cover the following main topics: chemistry and mechanism of action, experimental evaluation, phase I and II studies, and phase III studies.

For further details, application forms, and abstract forms, please contact: Dr. A. M. Arnold, C.R.C. & Wessex Medical Oncology Unit, Room C.F. 93, Southampton General Hospital, Southampton S09 4XY, England.

Thirteenth Meeting of the International Society of Pediatric Oncology (SIOP)

Marseille, France, September 15–19, 1981

"Tumors of the Neural Crest" will be the main theme of the Thirteenth Meeting of the International Society of Pediatric Oncology, which will be held September 15–19, 1981, at the Hôpital D'Enfants de la Timone, Marseille, France. The meeting will be conducted in English. Topics to be discussed include neuroblastoma, pheochromocytoma, melanoma, malignant degeneration in neurofibromatosis, medullary thyroid carcinoma, and other related tumors. Current trials on Wilms' tumor, osteosarcoma, rhabdomyosarcoma, and medulloblastoma will be reported. There will also be a pathologist's meeting. The organizing committee includes C. Raybaud, R. Clement, G. Lebreuil, and J. L. Bernard.

For more information, contact: Pr. C. Raybaud, 13th SIOP Meeting, Hôpital d'Enfants de la Timone, 13385 Marseille Cedex 4 France.

Sixth Annual Symposium on Advances in Cancer Treatment Research

Baltimore, Maryland, September 24–26, 1981

The Sixth Annual Symposium on Advances in Cancer Treatment Research will be held September 24–26, 1981, at the Baltimore Convention Center, Baltimore, Maryland. *For additional information, contact*: Program of Continuing Education, University of Maryland School of Medicine, Rm 300, MSTF, 10 S Pine Street, Baltimore, MD 21201 (301-528-3956).

International Symposium on Endocrinology of Cystic Breast Disease

Turin, Italy, November 5–6, 1981

An International Symposium on Endocrinology of Cystic Breast Disease will be held November 5–6, 1981, in Turin, Italy. The symposium is being organized by Clinica Medica B, University of Turin, under the auspices of the Italian Society of Endocrinology. Topics of interest to both patient-oriented investigators and biochemists of the mammary gland will be discussed: breast physiology, pathology of benign breast disease, hormone receptors in benign breast disease, biochemistry of breast cyst fluid, endocrinologic patterns in cystic breast disease, and clinical and therapeutic aspects.

The program will include invited lectures and a limited number of short communications. The official languages will be English and Italian.

Invited speakers are A. Angeli (Italy), H. L. Bradlow (US), G. Bussolati (Italy), R. Di Carlo (Italy), L. Dogliotti (Italy), P. Fioretti (Italy), D. E. Haagensen, Jr (US), F. Halberg (US), J. Lancranjan (Switzerland), P. Mauvais-Jarvis (France), C. Robyn (Belgium), P. H. Rolland (France), U. Veronesi (Italy), and C. Welsch (US).

For additional information and registration forms, contact: Scientific Secretariats A. Angeli and L. Dogliotti, Clinica Medica B, Università di Torino, Via Genova 3, 10126 Torino, Italy. Tel (011)690.654/637.230.

Fourth Annual San Antonio Breast Cancer Symposium

San Antonio, Texas, November 6–7, 1981

The University of Texas Health Science Center at San Antonio and the Cancer Therapy and Research Center of South Texas are sponsoring the Fourth Annual San Antonio

Breast Cancer Symposium on November 6–7, 1981, in San Antonio, Texas. The symposium is accredited by the American Medical Association. Proffered papers on the experimental biology, etiology, prevention, diagnosis, and therapy of breast cancer are invited. The deadline for abstracts is June 1. *For further information, contact*: Office of Continuing Education, University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Dr, San Antonio, TX 78284 (512-691-7295).

Current Concepts in Cancer Therapy

Saint Louis, Missouri, December 10–12, 1981

A program entitled "Current Concepts in Cancer Therapy" will be held December 10–12, 1981, in Saint Louis, Missouri. The Program Chairman is Marc K. Wallack, MD. The conference is being presented by the Section of Surgical Oncology, Department of Surgery, Washington University in Saint Louis and the Missouri Chapter of the American Cancer Society. The American Medical Association, American Academy of Family Physicians, and American Osteopathic Association have approved 19 credit hours for this program.

For information, contact: Office of Continuing Medical Education, Washington University School of Medicine, Box 8063, 660 S Euclid Ave, Saint Louis, MO 63110 (314-454-3873).

Third University of Arizona Cancer Center Conference on Human Tumor Cloning

Tucson, Arizona, January 10–12, 1982

The Third University of Arizona Cancer Center Conference on Human Tumor Cloning will be held January 10–12, 1982, at the Arizona Health Sciences Center, Tucson, Arizona. Conference co-chairmen are Sydney E. Salmon, MD, and Jeffrey M. Trent, PhD. This third conference on human tumor cloning will follow a different format than those held previously. On January 10, an optional basic "primer" course (including wet laboratory) will be held. On January 11–12, a combination of invited and competitively selected papers will be presented by established investigators in the field. The deadline for submission of abstracts is September 14, 1981.

For information on registration and abstracts, please contact: Mary Humphrey, University of Arizona Cancer Center, Tucson, AZ 85724 (602-626-6044).

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